Prevention of short- and long-term complications in dialysis patients: The role of predilution on-line hemofiltration'.

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ISBN 90-5278483-3

Cover design: 'Ninpheas Et Pont Japonais' by Claude Monet
Layout: Tiny Wouters
Production: Datawyse | Universitaire Pers Maastricht

Printing of this thesis was financially supported by: Amgen B.V., Baxter B.V., Fresenius Medical Care, Gambro B.V., Genzyme Nederland, Hospal B.V. and Janssen-Cilag B.V.

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PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof.mr. G.P.M.F. Mols,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 23 september 2005 om 16.00 uur

door

Charles Beerenhout

UNIVERSITAIRE PERS MAASTRICHT

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Chapter 1

Introduction

Introduction

Historic overview

Removal of toxins from the human body by blood purification has an ancient history. Before modern times, blood purification techniques were not performed in order to treat a specific organ disease, but to restore the overall balance within the body. Apart from enemas and sweating baths, blood letting was a common practice in ancient Greco/Roman medicine to restore the balance of the four humors (blood, phlegm, black and yellow bile). The disequilibrium of these four humors was considered the main cause of diseases and restoring the balance should contribute to health. The theory of the four humors was already described by Hippocrates (460-377 BC), and strongly supported by the authority of Galen (131-201 AD). The humoral theory remained the basis of medicine throughout the Middle Ages and renaissance. Even after the emergence of more scientifically based medicine in the 17th century these ancient theory still sustained authority and the practice of blood letting has been widely performed till the mid 19th century.
In the 19th century, the concepts of health and disease underwent great changes in western medicine. Like in chemistry and physics, the ancient philosophy-oriented approach was replaced by a science-oriented approach based on pathophysiology. This important change in medical approach led to the near abandonment of blood letting as means for blood purification.
In the mid 19th century experiments lead to a better understanding of the specific organs in maintaining an internal balance or homeostasis. Richard Bright, English physician (1789-1858) was the first to understand the relation between failing kidney function and the clinical picture of "dropsy". The French physiologist Claude Bernard (1813-1878) described the role of the different organs in the maintenance of the internal milieu of the body. The kidney was identified as a major organ in the removal of toxins and fluid. Malfunction of the kidney led to disease and finally death. Despite the evolving knowledge on renal physiology and pathology an adequate treatment of kidney failure was not available.

A scientific breakthrough in blood purification came with the experiments of Scottish chemist Thomas Graham (1805-1869), who was the first to show diffusion of water-soluble substances over a membrane. It took until the first part of the 20th century before the possible role of diffusion over a membrane as a treatment of renal failure was recognized. The first (unsuccessful) dialysis treatment in humans was performed by German physician Georg Haas (1886-1971). More successful were the Dutch physician Wilhelm Kolff (born 1911) and the Swedish physician Alwall (1906-1986)[1]. Kolff was the first to keep a

patient with acute renal failure alive with dialysis treatment (1945)[2]. The artificial kidney constructed by Kolff consisted of cellophane tubing wrapped around a steel drum, in which blood was entered. The steel drum was placed in a saline bath, after which urea was removed by means of diffusion. Because of the unavailability at that time of a long lasting vascular access, was hemodialysis only used in the treatment of acute renal failure. After the introduction of the arteriovenous fistula by William Scribner and colleagues in the early sixties hemodialysis became more available as a treatment for patients with chronic renal failure.
A parallel development occurred with the introduction of peritoneal dialysis. Although, it was after the introduction of an indwelling catheter, that peritoneal dialysis became available as a routine treatment in the seventies[1].

The present situation

Nowadays, hemodialysis and peritoneal dialysis are widely performed in the treatment of patients with renal disease. In these patients, the native kidney function, in order to maintain the internal milieu, is insufficient. Blood purification techniques, such as hemodialysis and peritoneal dialysis, support restoring the internal milieu of the body by excreting toxic waste products and removal of excess fluid. The basic principle of both hemodialysis and peritoneal dialysis resides in the correction of the internal milieu by means of diffusion of dissolved substances through a semi-permeable membrane (figure 1.1). In hemodialysis the membrane separates the blood compartment in the artificial kidney from the dialysate compartment, through which there is a continuous flow of a purified electrolyte solution (dialysate). Removal of excess fluid occurs by applying of a negative pressure gradient between the blood and dialysate compartiment. In peritoneal dialysis, the peritoneal interstitium, consisting in capillaries and mesothelium, serves as the semi-permeable membrane. Diffusion takes place between blood and a dialysate solution, which is installed in the peritoneal membrane through a catheter. Removal of excess fluid occurs by application of an osmotic pressure gradient by addition of a hypertonic fluid to the dialysate solution.

Despite the fact that death from end-stage renal failure per se has become rare in the Western world, morbidity and mortality is remarkably higher in dialysis patients compared to the overall population[3]. Moreover, present renal replacement therapies are far from optimal. In contrast to the native kidney, hemodialysis is performed at an intermittent basis, which leads to un-physiologic fluctuations in the internal milieu. Whereas peritoneal dialysis is performed at a continuous basis, its efficacy in removing uremic waste products is also far less compared to the healthy kidney.

Another aspect is the nature of the removal of toxic waste products by dialysis compared to the normal filtration by the native kidney. Although diffusion, on which hemodialysis and peritoneal dialysis are based, is a very efficient process for clearance of small molecules, the clearance of larger molecules is far less efficient. This is due to the inherent nature of the diffusive process itself, based on random movement of dissolved substances, which is less for larger molecules[4]. Moreover, the permeability of the most commonly used (so-called low-flux) hemodialysis membranes for larger molecules is relatively low. Although membranes used for hemodialysis should be impermeable for substances with the molecular weight of albumin (±58 kD) and higher, the cut-off level (above which there is no transmembranous transport of middle molecules possible) of most low-flux membranes is ±5 kD. However, also the transport of larger molecular weight molecules with molecular weights below 5 kD is limited, as transmembranous transport progressively decreases in relation to molecular weight, even below the cut-off point of the membrane.
The use of more permeable, so-called high-flux, membranes (cut-off level ±55 kD), results in a larger removal of larger molecules, which is however still limited due to the nature of the diffusive process[4-6].
It has been suggested that the insufficient removal of larger molecules is related to the poor outcome in patients on dialysis[7,8]. However, as will be discussed below, there is still a scarcity of information about the patho-physiological effects of accumulation of larger molecular weight toxins.
Larger substances can be more efficiently removed by hemofiltration, which is a dialysis treatment based upon convection instead of diffusion. With hemo-filtration, plasma water is removed (filtrated) by means of a pressure gradient over an artificial membrane (figure 1.1), with concordant removal of dissolved substances (solvent drag). Removal of uremic toxins is dependent upon the filtration volume and the permeability of the membrane. In hemofiltration, high permeability membranes (cut-off level ±55 kD) are commonly used. The removed fluid is replaced by infusion of substitution fluid with approximately the same concentration of electrolytes and bases as dialysate[9].
Hemofiltration was first introduced in the seventies by Quellhorst and Henderson[1]. Hemofiltration can be performed as a purely convective technique, or in combination with diffusion, hemodiafiltration, which will increase smaller molecular clearance[9].
After a burst of initial attention supported by promising results in patients, enthusiasm for hemofiltration declined during the mid-eighties. Probably both because for economical motives due to the high costs of the pharmaceutically prepared substitution fluid and of the limited removal of small molecules in convection compared to diffusive techniques.
By on-line preparation of substitution fluid from reverse osmosis water and dialysate concentrates, cleansed by multiple filtration steps, it has become

possible to produce large amounts of substitution fluids in an economically feasible way[10]. By the use of higher volumes of substitution fluids, it also has become possible to circumvent the problem of the lower small molecular clearance. These developments lead to a renewed interest in convective treatments as hemofiltration and hemodiafiltration, and on-line filtration techniques have been introduced in various European countries. As will be discussed below, convective therapies have been advocated both for the prevention of short-term and long-term complications in dialysis patients.
During hemofiltration, the clearance of uremic toxins is determined by the filtration volume and the permeability of the high flux membrane in relation to the molecular weight of the substance. Thus, when the passage of a solute is not limited by the permeability characteristics of the artificial membrane, its removal is basically equal to the filtrated volume.

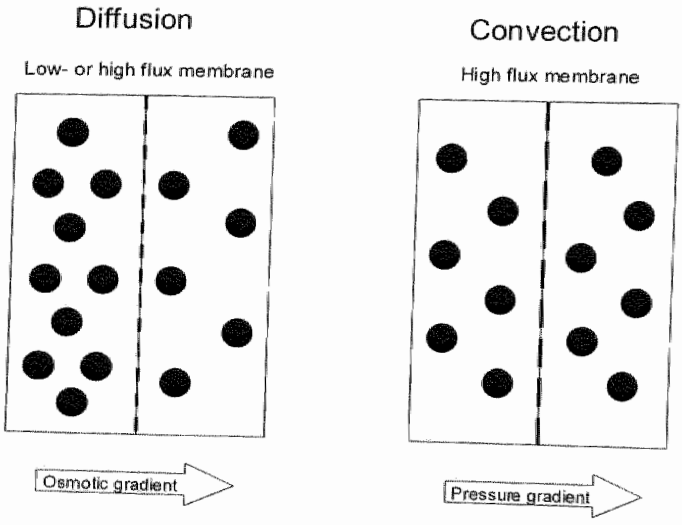

Figure 1.1 Mechanism of diffusion and convection.

Technical aspects of convective clearance

During hemofiltration, substitution fluids have to be infused to replace the fluid removed from the patient. This infusion can take place before (pre-dilution) or after the artificial membrane (post-dilution). With the pre-dilution approach, plasma water is diluted before entering the artificial kidney usually in a 1:1 ratio (figure 1.2). Thus, in principle, solute clearance is 50% of the filtrated volume. However, the filtration volume is only limited by the transmembranous pressure in the artificial kidney and by the capacity of the hemofiltration module. In

clinical practice, it is often possible to achieve filtration rates up to 300-400 ml/min with pre-dilution hemofiltration (clearance 150-200 ml/min for substances with unrestricted passage through the artificial membrane).

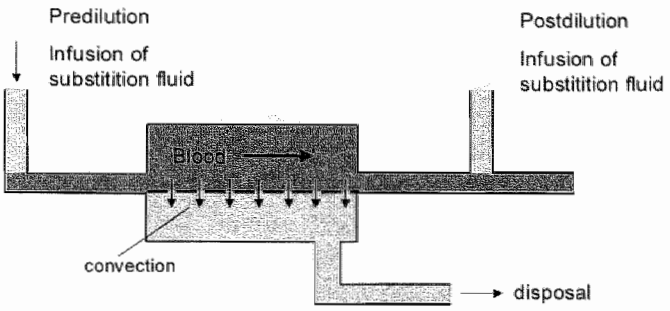

Figure 1.2 Pre versus postdilution infusion of substitution fluid.

In the post-dilution approach, filtration volumes are more limited due to hemoconcentration in the artificial kidney. In general, filtration is limited to 25-33% of the blood flow rate (most commonly set between 300-400 ml/min)[10]. Thus, clearance during post-dilution hemofiltration will generally not exceed 125 ml/min, which leads to insufficient small molecular removal compared to hemodialysis.

During hemodiafiltration, the clearance of small molecules is in general higher compared to hemofiltration. However, during hemodiafiltration, the pre-dilution mode, which would permit the highest middle molecular clearance due to the allowance of higher filtration rates, has the theoretical disadvantage that blood becomes less concentrated, reducing the diffusion gradient and thus small molecular clearance compared to post-dilution hemodiafiltration. In general, the difference in small molecular clearance between pre- and post dilution appears to be larger when higher filtration rates are used[10]. In comparative studies, the difference in urea clearance between pre- and post-dilution hemodiafiltration differed between 2 and 17 ml/min[11,12].

In general, the post-dilution mode is advocated for hemodiafiltration[9]. Convective clearance during post-dilution hemodiafiltration is affected by the same factors as discussed above for post-dilution hemofiltration.

Interestingly, also during high-flux hemodialysis some convective clearance takes place due to a pressure drop in the blood compartment of the artificial membrane. However, in general, the convective clearance during this so-called backfiltration will not exceed 30 ml/min[13].

Summarizing, with the techniques presently available, the clearance of larger molecular weight uremic toxins appears to be highest during pre-dilution hemofiltration. With this approach, clearance of smaller molecular weight solutes will be nearly comparable to that achieved during hemodialysis.

Safety aspects

Substitution fluid used in convective treatments is infused directly into the patient without separation by an artificial membrane. The substitution fluid has to meet therefore the strictest microbiological criteria for sterility. The sterility of the substitution fluid is achieved by using ultra filters. Contaminants are cleared both by means of restricted passage trough the ultra filter, as well by absorption unto the filter. The passage of endotoxin fragments, which are below the cut-off point from the filter, is restricted. Filters used for water treatment in convective treatments are able to reduce bacteria and endotoxin fragments by at least 10^7 and 10^3, respectively[10].

Despite similarities with regard to the principle of "cold sterilization", the two main commercially available systems differ in the handling of the incoming water. The Fresenius® ONLINE plus™ system uses two ultra filters, the first is placed after the proportioning system and the second, which serves as a safeguard in case of loss of integrity of the first filter, is placed before the substitution port. The two ultra filters are replaced after a definite time period and are tested for integrity before each treatment[14].

The Gambro® AK-200 ULTRA system uses three ultra filters (figure 1.3), the first filters the incoming water, the second is placed after the proportioning system, and the third just before infusion into the patient. The final filter is replaced after every treatment; the other filters are replaced after a definite time period.

Both manufacturers state that, when pre-treated (reverses osmosis) water is used according to AAMI (American association medical instruments) standards, sterility of the final water is ensured. This is also stated in the CE (Certification European)-marking of the devices used for on-line hemofiltration[10]. This European guideline is not followed by every state, due to different opinions regarding the substitution fluids. In the Netherlands, substitution fluids are considered as pharmaceutics compounds and therefore have to comply with the European Pharmacopoeia instead of the standard guideline. Recently, a guideline for the production and surveillance of substitution fluid for on-line filtration techniques was produced by the Dutch Federation of Nephrology in collaboration with the National Health Inspection.

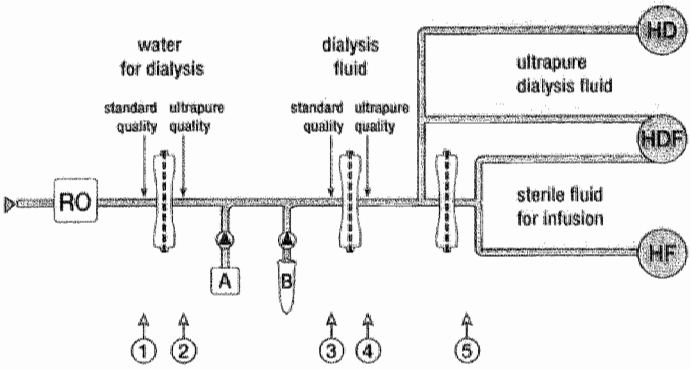

Figure 1.3 Filter placement in the Gambro® AK-200 ULTRA system.

Costs

Convective dialysis techniques as hemodiafiltration and hemofiltration, using on-line substitution fluids, are more expensive than diffusive dialysis therapies. It should be taken into account that also the use of high-flux artificial membranes and ultra pure dialysate is much more expensive than conventional dialysis using low-flux membranes and water of standard bacteriological quality. Extra costs for on-line therapies above conventional low-flux dialysis include the use of larger artificial membranes, additional ultra filters and infusion lines. More additional costs are due to cultures and endotoxin determinations and more sophisticated disinfections procedures of the dialysis module. Roughly calculated, not taking into account the additional costs for monitoring of fluids, the extra costs for one on-line filtration treatment would be approximately 6-21 Euro compared to high-flux diffusive dialysis and 17 to 32 Euro compared to conventional low-flux dialysis. All these figures of course depending upon local conditions.

Classification of uremic toxins

By the normal kidney, substances up to 58 kD (the molecular weight of albumin) are removed, these substances accumulate in renal failure. In the literature, uremic toxins are often divided into small, middle and larger molecular weight uremic toxins. Smaller molecular weight uremic toxins (<300 Dalton), such as urea and creatinine are very efficiently removed by hemodialysis. It is clear that the sequels of the uremic syndrome cannot be explained only by the accumulation of smaller molecules like urea, which is relatively a non-toxic molecule[15].

Uremic toxins above 300 D are often sub classified as "middle" and "large" molecular weight uremic toxin. However, the definition of "middle" molecules according to molecular weight is not straightforward in the literature and varies from 300-12000 D, 500-15000 D, or 500-2000 D[16-19]. Therefore, in this thesis, the distinction between middle and large molecules will be used as little as possible.

With regard to the accumulation of uremic solutes in patients treated with hemodialysis, it is important to consider that several smaller molecular weight uremic toxins, such as p-cresol, indoxyl sulphate, hippuric acid, homocysteine, and possibly L-asymmetric-dimethylarginine (L-ADMA) may behave like larger molecular weight substances due to protein binding, molecular configuration, or electrostatic charges[20].

ß2-microglobulin is the most well-known and studied larger molecular weight uremic toxin (12 kD) and has been implicated in the development of dialysis related amyloidosis[21]. As for larger molecules, removal rates of β-2 microglobulin appear to be approximately 25-30% higher during on-line filtration techniques compared with high-flux hemodialysis[22-24]. It was shown that β-2 microglobulin clearance during hemofiltration exceeded the clearance during high-flux hemodialysis already at filtration rates above 60 ml/min[24].

There is also evidence that other larger molecular weight molecules, which are insufficiently removed by hemodialysis or peritoneal dialysis treatment, may play a role in chronic complications in dialysis patients[25]. Moreover, convective therapies may also have a beneficial role in the prevention of acute complications occurring during the dialysis treatment itself.

Short-term complications of dialysis treatment and possible benefits of convective treatments

Hypotension is the most common acute complication during dialysis treatments. Especially in vulnerable patients, such as those with pre-existent cardiac or cerebrovascular disease, hypotension during dialysis may even ensue in serious complications, such as myocardial or cerebral ischemia.

The pathophysiology of hemodynamic instability is multifactorial. During intermittent dialysis therapies, fluid which has accumulated in the interdialytic period (i.e., 2 to 3 days) has to be removed. Depending on the dietary compliance of the patient and the residual renal function, between 1 and 3 liters of fluid have to be removed in a relatively short period (i.e., 3 to 5 hours), which will lead to a decline in blood volume. This decline in blood volume is dependent upon the amount of fluid removed by ultrafiltration and upon the refill from the interstitium[26]. The refill from the interstitium is dependent upon many factors, such as the hydration state of the patient and the sodium concentration in the dialysate (in case of hemodialysis) c.q. substitution fluid (in

case of hemofiltration). With an increased sodium concentration in dialysis/substitution fluid, more fluid will be removed from intracellular to the extra cellular spaces and subsequently the refill of blood volume from the tissues is enhanced. However, a reduced sodium removal may have untoward long-term effects in view of increased thirst, inter-dialytic weight gain and hypertension.

It has been suggested that the decline in blood volume is less during hemofiltration compared to hemodialysis, which has been related to a reduced sodium removal during hemofiltration. This might be explained by increased coating of negatively loaded proteins to the artificial membrane due to hemoconcentration. There is however uncertainty whether this effect is present both during pre- and post-dilution hemofiltration[27,28]. During pre-dilution on-line hemofiltration, protein clotting to the membrane may be less pronounced due to dilution of the blood before entering the membrane[27].

Convective therapies may also have an effect on the vascular response during dialysis treatments. The physiological response to a decline in blood volume is a constriction of the resistance (i.e., arterioles) and capacitance (i.e., veins and venules) blood vessels. Constriction of the capacitance vessels leads to mobilizing unstressed blood volume from the peripheral to the central compartments, thus maintaining cardiac output. During conventional hemodialysis, the constriction of the capacitance and resistance vessels was found to be inadequate, leading to insufficient mobilization of hemodynamically inactive (unstressed) blood[26]. Different explanations have been sought to explain the pathophysiology of this phenomenon. Firstly, during hemodialysis with commonly prescribed dialysate temperatures of 37-37.5°C, core temperature increases, leading to vasodilation in the cutaneous blood vessels, which will counteract the hemodynamic response to hypovolemia[29]. Another explanation resides in the generation of nitric oxide (NO), a potent vasodilator during dialysis[30]. Interestingly, NO release may be influenced by changes in blood temperature[31], although information on the relation between dialysis, NO synthesis and blood temperature is very limited in the literature[32].

It is since long known that vascular reactivity is better maintained during convective treatments compared to hemodialysis with commonly used dialysate temperatures of 37-37.5°C[33]. The difference in hemodynamic response between diffusive and convective treatments appears to be mainly based on differences in vascular reactivity. Several explanations have been proposed for this phenomenon. Firstly, during conventional hemofiltration with pharmaceutically prepared substitition fluid, the temperature of the infusate is generally below 37°C and thus the increase in core temperature is prevented[34,35]. However, it has also been suggested that an increased removal of higher molecular weight vasodilating substances, such as calcitonin related

gene peptide, might account for the superior hemodynamic response during hemofiltration[36,37].

This hypothesis seems to be supported by hemodynamic data obtained during on-line hemofiltration, during which also a decreased prevalence of hypotensive episodes was observed compared to hemodialysis with an equal temperature of respectively substitution fluid and dialysate[37].

However, the thermal effects of on-line therapies may not be entirely comparable to those of hemodialysis, even when the temperature of substitution fluid and hemodialysis are equal. Data on the thermal effects of on-line convective therapies are limited. However, in one study, the response of the forearm vascular bed between hemodialysis and on-line hemofiltration was found to be equal after meticulous control of thermal factors[38].

Pre-dilution hemofiltration with the possibility of archiving high filtratation volumes, represents an interesting model to study the acute hemodynamic effects of increased convective clearance per se.

Long-term complications in dialysis patients and potential benefits of convective therapies

More than 50% of total mortality in dialysis patients is caused by cardiovascular disease[39], which is therefore the leading cause of death in this population. In younger dialysis patients, the mortality of cardiovascular disease is more than 100 fold increased compared to the general population[3]. Apart from classical risk factors, also more specific factors related to the renal disease or dialysis treatment may contribute to this increase in mortality[39].

Hypertension, which is present in a large percentage of dialysis patients, is an important risk factor for cardiovascular disease in dialysis patients[40]. It is also a major determinant for the development of structural abnormalities of the heart and blood vessels, such as left ventricular hypertrophy (LVH) and an increase in arterial stiffness, which are itself independent risk factors for mortality in dialysis patients[41,42]. The pathogenesis of hypertension in dialysis patients is determined by various factors, such as accumulation of extra cellular fluid, and an inappropriate activation of the renin-angiotensin-aldosteron (RAAS) and sympathetic nervous systems[43]. Recently, accumulation of the endogenous NO synthase inhibitor: l-asymmetric dimethylarginine (l-ADMA) in dialysis patients has been proposed as a pathogenetic factor for both hypertension and structural cardiovascular abnormalities[44]. Data on the effect of convective therapies on l-ADMA removal, which has a relatively small molecular weight (202 D) but behaves kinetically as a larger molecular weight substance are limited[45]. One study found a reduction in l-ADMA levels and an improvement in blood pressure control during on-line hemodiafiltration compared to low-flux hemodialysis[46].

It has also been proposed that accumulation of advanced glycation end-products (AGEs) in uremia might play a role in the pathogenesis of cardiovascular abnormalities in dialysis patients. Accumulation of the AGE product carboxymethyllysine was observed in the vascular wall of uremic patients[47]. Moreover, AGEs may also play a role in endothelial dysfunction and generate inflammatory products in dialysis patients[48,49]. Data on the effect of different dialysis strategies on the removal of AGEs are limited, which is also due to the fact that various AGE compounds are not well characterized[50]. AGE removal was found to be larger during on-line hemodiafiltration compared to high-flux hemodialysis[46,51]. However, in another study, pre-dialytic levels of carboxymethyllysine did not differ between patients treated with hemodialysis and on-line hemodiafiltration[52]. Part of the discrepancies may be explained by the fact that also purity of the dialysate may play a role in AGE formation. Thus, the effect of an increased convective clearance on AGE levels would best be studied in comparison with hemodialysis with ultrapure dialysate.

Hyperhomocysteinemia is also supposed to play a role in the increased cardiovascular morbidity in dialysis patients[53]. Homocysteine accumulates in dialysis patients but was shown to be removed by high-flux dialysis therapies[54]. Regarding clinical effects, retrospective studies showed superior results of convective techniques in view of hypertension control and cardiovascular morbidity compared to hemodialysis[55,56]. There is also preliminary evidence that the use of convective therapies may result in an improvement in cardiac structure[57]. Moreover, an increased removal of middle molecules was inversely related to mortality in dialysis patients[8]. On the other hand, results of the recently published HEMO study did not show a beneficial effect of high-flux compared to low-flux hemodialysis on overall survival, although cardiac death appeared to be decreased slightly[58]. It should be stressed however that the clearance of larger molecular weight solutes during pre-dilution hemofiltration is higher compared with high-flux hemodialysis. However until now the potential beneficial cardiovascular effects of pre-dilution hemofiltration have not yet been studied in a prospective, controlled study.

Another factor that is strongly related to mortality in dialysis patients is an impaired nutritional state[59]. The incidence of malnutrition is high in dialysis patients, whereas markers of body protein stores were found to decline even further with increasing time on dialysis[60]. Although its pathogenesis is multifactor, a reduced appetite appears to be a major contributing factor to the impairment of nutritional state in dialysis patients[61]. This suppression of appetite might be related to an accumulation of middle-molecular substances. In an animal model anorexia could be induced by ingestion of a mixture of as yet unidentified uremic toxins with a molecular weight range varying between 2 and 5 kD[62]. Moreover, a possible role for leptin (16 kD) as an appetite-suppressing factor in dialysis patients has been suggested. Leptin is a

substance produced in adipocytes, which physiological decreases appetite through a direct effect on the hypothalamus[63]. Patients on dialysis treatment have an increased serum leptin level compared with controls[64]. Leptin is not removed by low-flux hemodialysis, whereas during high-flux dialysis, a reduction in serum leptin levels was observed[6]. Thus, due to the superior removal of larger molecular weight substances during pre-dilution hemofiltration, an improvement in nutritional state may be expected. Data regarding the effect of convective therapies on nutritional state in literature are very scarce.

Lastly, many dialysis patients are in a state of inflammation, which is a strong risk factor for mortality and may also play a role in the pathogenesis of cardiovascular complications and malnutrition[65]. The pathogenesis of the inflammatory state in dialysis patients is multifactorial. An increased prevalence of infectious complications, partly explained by the immune dysfunction in dialysis patients, may play a role in this aspect. A substances of potential interest is complement factor-D (24 kD), which activates the alternative pathway of complement, but also inhibits the degranulation of poly-morphonuclear granulocytes[66]. Complement factor-D decreased during on-line hemodiafiltration compared to high flux dialysis[22].

Rationale for the thesis

Convective therapies with on-line production of substitution fluid offer the opportunity for as yet unrivaled larger molecular clearance in an economically feasible way. The highest larger molecular weight clearance can be achieved with pre-dilution hemofiltration. Moreover, pre-dilution hemofiltration represents an interesting model for studying the pathophysiological effects of accumulation of larger molecular weight substances in patients with end stage renal disease (ESRD).

Whereas the beneficial effects of hemofiltration on the prevention of hemodynamic instability is beyond discussion, there is still debate in the literature about the pathophysiological mechanisms behind this phenomenon. It has been proposed that a less decline in blood volume, due to less sodium removal during hemofiltration, by increased coating of negatively loaded proteins to the artificial membrane, may partly account for this phenomenon[28,67]. However, few studies have focused on sodium removal during pre-dilution on-line hemofiltration, during which protein coating to the artificial membrane may be less pronounced.

Also the constriction of resistance and capacitance vessels during a decline in blood volume is more physiological during hemofiltration[33,36,38,68]. It has been suggested that either temperature related factors or an increased removal of vasodilating substances during hemofiltration might account for this

phenomenon[34-38]. However, little is known about the thermal effects of on-line convective therapies. Moreover, few studies exist in which the hemodynamic effects between hemodialysis and on-line convective treatments, matched for thermal factors, were compared. Interestingly, experimental data suggest that thermal factors may also influence the synthesis of NO, which was also found to contribute to the impaired vascular response during hemodialysis. However, in-vivo data on this phenomenon are very limited[30-32].

On-line convective therapies, such as pre-dilution on-line hemofiltration, may also have a beneficial effect on various long-term complications in dialysis patients due to an increased removal of larger molecular weight substances. Data from experimental and uncontrolled studies suggest that an increased removal of larger molecular weight substances may result in an improvement of cardiovascular and nutritional parameters, and possibly a modification of the inflammatory state in dialysis patients[55-58,62,63,68]. However, randomized studies with filtration therapies are scarce and did not focus on cardiovascular and nutritional parameters in detail[22,69].
Several complications in dialysis patients may be unrelated to the dialysis treatment per se and may even be difficult to modify. In this aspect, the origin of the renal disease itself may be related to cardiovascular complications. It may be hypothesized that, due to the generalized diseased process, cardiovascular abnormalities may be more pronounced in patients with diabetes mellitus or nephrosclerosis/renal vascular disease as the cause of renal failure[70]. However, this aspect has received little attention in the literature. Regarding the greatly higher prevalence of inflammation in dialysis patients, it is not well known to what extent this inflammatory process varies in dialysis patients and to which degree it is related to co-morbid events.

Outline of the thesis

The first part of the thesis focused on short-term complications during dialysis treatment.
In chapter 2, the thermal effects of hemodialysis and on-line convective therapies were studied in-vitro. Subsequently, in chapter 3, the hemodynamic response during pre-dilution hemofiltration and hemodialysis with different temperatures of dialysate was assessed in vivo. In chapter 4, the effect of temperature of dialysate on nitric oxide synthesis in relation to hemodynamic parameters during standard dialysis was studied.

The second part of the thesis focused on long-term complications in dialysis patients and the potential benefits of pre-dilution on-line hemofiltration. In

chapter 5, the results of a randomized study comparing the effects of pre-dilution on-line hemofiltration and low-flux hemodialysis on the cardiovascular and nutritional state of dialysis patients, as well as their respective effects on quality of life and the uremic toxicity profile are reported. In chapter 6, it was investigated to what extent not modifiable factors, such as age and the cause of renal failure itself, were related to an increased vascular stiffness, an important risk factor for mortality. In chapter 7, the relation between co-morbid events and the inflammatory state was investigated.
In chapter 8, the results of the thesis were put into perspective with data from the literature.

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Chapter 2

Thermal effects of different dialysis techniques and blood pump speeds: an in vitro study

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Journal of Nephrology 2003;16:552-7

Abstract

Background

Haemodynamic stability is improved during convective dialysis treatments compared with standard haemodialysis. Thermal effects have a pivotal haemodynamic impact on hemodynamic stability during dialysis procedures. In contrast to conventional dialysis techniques, no data are present in the literature regarding the thermal energy balance during on-line convective techniques. Secondly, little data exist on the effect of extracorporeal blood pump speed on thermal energy balance.

Methods

In the present study firstly relative differences in energy transfer rate (ETR) over the extracorporeal circuit was assessed during on-line hemo(dia)filtration (H(D)F) procedures (infusate temperature 37.5°C) and hemodialysis at different dialysate temperatures during an in-vitro procedure using a blood temperature monitor (BTM®) whereas in the second part of the study the thermal effects of different blood pump speed rates was assessed during the various treatment modalities.

Results

ETR was different between all treatment modalities ($p<0.05$) studied, except for hemodialysis at 36.5°C versus pre-dilution HF and post-dilution HDF versus hemodialysis at 37.5°C. ETR was most negative, indicating the largest energy loss, during hemodialysis at 35.5°C (-58.5.2±2.6 W), whereas it was nearly comparable between pre-dilution HF (-30.7±4.1W) and hemodialysis at 36.5°C (-35.1.2±2.4 W). Post-dilution HDF (-17.7±1.2 W) resulted in an ETR comparable to that of hemodialysis at 37.5°C. (-15.0±3.9 W). ETR during post-dilution HF was –43.8±1.3 W. The thermal effect of the blood pump speed was most pronounced during the procedures with the more negative energy transfer rates.

Conclusion

When studying hemodynamic stability between different treatment techniques, the thermal effects of the procedure and of the blood pump speed should be considered.

Introduction

Since the pioneering work of Maggiore et al. in the early eighties, it is well known that the extracorporeal blood temperature has a pivotal impact on hemodynamic stability during dialysis procedures, which is mainly due to the effect of thermal factors on vascular reactivity[1-3]. More recent studies have confimred that the energy transfer over the extracorporeal system is the primary, if not the single, explanatory variable for differences in vascular reactivity during various dialysis techniques[4-6]. Still, especially in view of the increased interest for on-line hemo(dia)filtration techniques, there is still discussion in the literature about the potential role of convection per se on hemodynamic stability during these treatment modalities[7]. However, in contrast to conventional dialysis techniques, no data are present in the literature regarding the energy transfer rate (ETR) during on-line techniques. It can be hypothesized that, even when the temperature of the dialysate is equal to that of the infusate, pre-dilution on-line hemofiltration (HF) results in a more pronounced energy loss compared to hemodialysis, due to the absence of heat transfer through the dialysis membrane (which also serves as heat exchanger). On the other hand, ETR during hemodiafiltration (HDF) and hemodialysis would be expected to be comparable. Still, as no studies exist on the relative differences in ETR between on-line H(D)F techniques and more conventional treatment modalities, experimental data with regard to this subject are needed, especially in view of further studies comparing hemodynamic stability between these different techniques.

The effect of extracorporeal blood pump speed on hemodynamic stability during dialysis has also not received much attention. From a theoretical point of view, blood pump speed and the difference between the temperature in the arterial and venous blood lines are the primary determinants of the ETR during the dialysis procedure[5,8]. It is to be expected that increasing blood pump speed leads to a reduction in the temperature gradient between the arterial and venous side of the extracorporeal circuit, due to a reduction in the exposure time of the blood to the cooler environment (i.e. when the blood passes the blood lines). On the other hand, as ETR is directly related to blood pump speed (see further), increasing blood pump speed could increase energy loss to the extracorporeal circuit, especially during treatment modalities with a large arteriovenous temperature gradient. The hypothesis to be tested is that the effect of blood pump speed on ETR is greatest in those treatments with the largest temperature gradient in the extracorporeal circuit.
Aims of the present study were firstly to compare the thermal effects of on-line H(D)F procedures with hemodialysis at different temperatures of the dialysate using an in-vitro procedure and secondly, to assess the influence of

extracorporeal blood pump speed on ETR during these different in-vitro procedures.

Methods

In this in-vitro study, a blood line (Fresenius®; Bad Homburg, Germany) was placed with an open end in a heated box filled with water (simulating the patient). Water in this box was kept at a constant temperature of 36.5°C by a heating module, which was monitored by constant temperature monitoring. This temperature of 36.5°C was chosen because it reflects the mean body temperature in dialysis patients, as found in clinical studies[5,6,9]. From the box, water was pumped through the blood line (simulating the arterial line of the extracorporeal circuit) to a polyamide filter (Polyflux 17S; Gambro®; Lund: Sweden) (Figure 2.1), attached to a Gambro AK-200 Ultra monitor, with which the different experimental treatments were performed. From the filter, the water was returned to the heating box by a second blood line (simulating the venous line of the extracorporeal circuit). Both the arterial and venous blood line were passed through a blood temperature monitor (BTM; Fresenius®; Bad Homburg; Germany), by which extracorporeal temperature and ETR can be assessed. During each treatment session temperature at the arterial (T_{art}) and venous (T_{ven}), as well as ETR between the extracorporeal circuit and the heated box were monitored at 10-second intervals. ETR was defined as the amount of thermal energy that was transferred from the extracorporeal circuit to the heated box, or vice versa. A positive value indicates net energy gain from the extracorporeal circuit to the heated box, and a negative value indicates net heat loss from the heated box to the extracorporeal circuit.

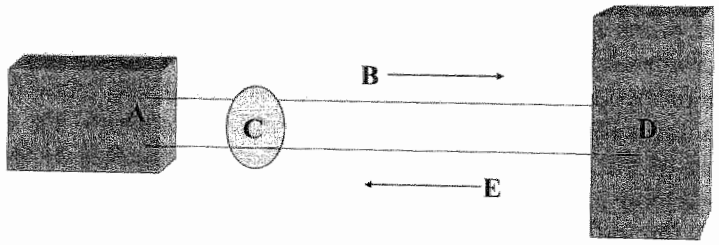

Figure 2.1 Schematic representation of the experimental situation.
A = heated box; B = blood line of the arterial part of the extracorporeal circuit; C = blood temperature monitor; D = dialysis module; E = blood line of the venous part of the extracorporeal circuit.

ETR (in Watts) is calculated by the BTM using the following formula:
$c \times \rho \times Qb \times (T_{ven}-T_{art})$, with c=specific thermal capacity (3.64 kJ/kg x °C), Qb=extracorporeal blood flow, ρ=density of blood (1052 kg/m^3)[8]. As the investigation was performed with water, the results obtained by BTM were multiplied by 1.09 [Density of water at is 994 kg/m^3 at 37°C, and specific thermal capacity of water is 4.2]. Hereafter values were converted in Watts (1W=3.6 kJ). T_{art} and T_{ven} were assessed with the use of continuous temperature monitoring at the arterial and venous side of the extracorporeal system by an air-filled head with a platinum sensor (Blood Temperature Monitoring (BTM®); Fresenius Medical Care; Bad Homburg; Germany) around the arterial and venous blood lines[5,8].
Because the BTM® automatically corrects for the heat capacity and density of the blood, values obtained in a watery environment cannot be expressed as absolute values. Nevertheless, because the correction factors for blood density and heat capacity of blood are constants, it is to be expected that BTM® can adequately detect relative differences in ETR.

First part

During the first part of the study, pre-dilution on-line HF (filtration volume 20 l/hr), post-dilution on-line HF (filtration volume 8 l/hr), post-dilution *HDF* (dialysate flow rate 625 ml/min, of which the filtration volume was respectively 4 and 8 l/hr), and hemodialysis at a dialysate temperature of respectively 35.5°C, 36.5°C, and 37.5°C, performed with the AK-200 Ultra monitor, were compared. The temperature of the infusion fluid and of the dialysate during the on-line HF procedures was set at 37.5°C, whereas the HDF sessions were studied with a dialysate/infusate temperature of 35.5°C and 37.5°C. The blood pump was set at 400 ml/min during all procedures. All measurements were performed five times on two different days. Values for ETR and temperatures in the arterial and venous line were obtained after a plateau had been reached. Ambient temperature was constant 22°C during the measurements.

Second part

Using the same technique as described above, during the second part of the study temperature in the arterial and venous side of the extracorporeal circuit and ETR were assessed during hemodialysis at 35.5°C, 36.5°C and 37.5°C, during post-dilution HF (8 l/hr) at 37.5°C, during postdilution on-line HDF (8 l/hr) at 37.5°C and during pre-dilution on-line HF (20 l/hr), using a blood pump speed of respectively 300 ml/min, 400 ml/min and 500 ml/min. Again, all measurements were performed five times.

Statistical analysis

Differences in ETR and arteriovenous temperature gradients between the different techniques and blood pump speeds were assessed by Friedman's ANOVA. If this analysis yielded significant results, differences were further analyzed with the Wilcoxon's signed rank test.

Results

First part

ETR was most negative, indicating the largest energy loss, during hemodialysis at 35.5°C (-58.5±2.6 W) and HDF (4 and 8 l/hr) at 35.5°C (-57.7±1.0 W respectively –57.8±0.8 W), whereas it was nearly comparable between pre-dilution HF at 37.5°C (-30.7±4.1W) and hemodialysis at 36.5°C (-35.1±2.4 W). Post-dilution HDF (4 and 8 l/hr) at 37.5°C (-13.9±2.7 W and -17.7±1.2 W) resulted in an ETR nearly comparable to that of hemodialysis at 37.5°C. (-15.0±3.9 W). ETR during post-dilution HF was -43.8±1.3 W. Results are displayed in figure 2.2. Data for HDF (4 l/hr) at 35.5°C, HDF (4 l/hr) at 37.5°C and HDF (8 l/hr) at 35.5°C are not graphically presented.
ETR was significantly different between all treatment modalities studied ($p<0.05$), except for hemodialysis at 36.5°C versus pre-dilution HF and post-dilution HDF (8 l/hr) versus hemodialysis at 37.5°C and between HD and HDF with a dialysate/infusate temperatures 35.5°C.

Second part

The effect of the blood pump speed on ETR was significant ($p<0.05$) during all treatment modalities. However, the effect of the blood pump speed was most pronounced during the procedure with the most negative energy transfer rates; i.e., hemodialysis at 35.5°C.
During all procedures, increasing blood pump speed reduced the temperature gradient significantly between the arterial and venous side of the extracorporeal circuit during the different procedures.

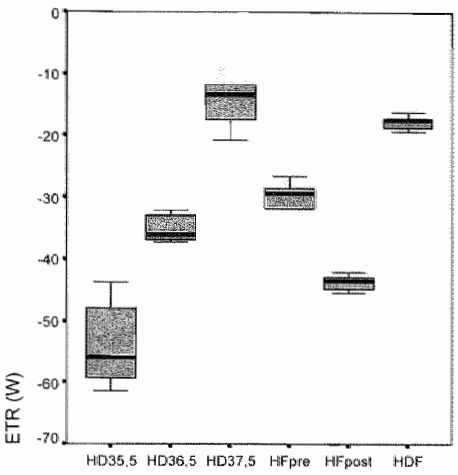

Figure 2.2 ETR during the various treatment modalities (blood pump speed 400 ml/min).
ETR = energy transfer rate; HD 35.5 = hemodialysis with dialysate temperature of 35.5°C; HD 36.5 = hemodialysis with dialysate temperature of 36.5°C; HD 37.5 = hemodialysis with dialysate temperature of 37.5°C; HF pre = pre-dilution on-line hemofiltration; HF post = post-dilution on-line hemofiltration; HDF = post-dilution on-line hemodiafiltration.
Values expressed as box-plots. Box indicates the 25th-75th percentile range (line in box=median). Capped bars indicate the 10th-90th percentile range.

Discussion

In the present study, different dialysis techniques were shown to differ largely with regard to extracorporeal ETR. Whereas this is well known for conventional dialysis techniques, this study is to our knowledge the first that compares the thermal effects of on-line H(D)F with conventional hemodialysis. In contrast to H(D)F with pharmaceutically prepared substitution fluid, which is generally stored at room temperature and therefore results in pronounced energy loss during the treatment[16], substitution fluid during on-line techniques is per definition heated to the same degree as the dialysis fluid. Nevertheless, pre-dilution on-line HF with an exchange volume of 20 l/hr resulted in an extracorporeal ETR that was comparable to that of hemodialysis with a dialysis temperature of 36.5°C. This is most probably explained by energy loss by both the blood and infusion lines and by the absence of heat gain over the dialysis membrane, which serves as a heat exchanger during conventional dialysis. Post-dilution HF resulted in an even larger energy loss to the extracorporeal circuit, which is probably explained by the fact that the amount of heated fluid

infused is less compared to the pre-dilution techniques. This difference in infusion volume between pre-and post-dilution HF reflects clinical reality and was chosen because, in contrast to pre-dilution HF, the amount of substitution fluid during post-dilution HF techniques is maximized by the extracorporeal blood pump speed[10]. As was to be expected from a theoretical point of view, post-dilution HDF had less cooling effect than both hemofiltration techniques, which can be explained by the effect that the extracorporeal blood is both heated by the dialysis and substitution fluid. ETR during post-dilution HDF resulted in a comparable ETR as hemodialysis with the same dialysate temperature, irrespective of the infusate volume. The absence of an effect of the infusate volume on ETR may be explained by the fact that the sum of the dialysate and infusate flow rate was set at 625 ml/min during all treatment modalities, which reflects clinical practice[10]. Thus, when the infusate flow rate is set higher, the dialysate flow rate is automatically lower and vice versa. Still, the fact that ETR during on-line HDF did not differ from hemodialysis needs to be explained in view of the additional energy loss from the infusate line to the environment. We believe that this additional heating loss may be counteracted because the sum of the dialysate and infusate flow rate (625 ml/min) during on-line HDF was higher than the dialysate flow rate (500 ml/min) during the hemodialysis session.

Both hemodialysis with a dialysate temperature of 37.5°C and post-dilution H(D)F resulted in an, albeit small, negative ETR. This is in line with earlier in vivo data and is likely explained by the energy loss from the blood lines to the environment[9].

In the second part of the study, the effect of extracorporeal blood pump speed on energy transfer rate was assessed during the different dialysis procedures. The findings of the present study are largely in agreement with the considerations of Schneditz et al[8]. From a theoretical point of view, the effect by which blood pump speed affects ETR might be opposite. On one hand, it increases the transfer of (cooled) blood from the extracorporeal circuit to the patient (the heating box). On the other hand it decreases the gradient between the temperature in the arterial and venous side of the extracorporeal circuit because of a reduction in energy loss to the environment[11], and might even generate thermal energy because of mechanical friction of the rollers on the arterial line. The present study showed that during the procedure with the most negative energy transfer, i.e. hemodialysis at 35.5°C, the effect of blood pump speed on extracorporeal ETR was most pronounced, despite the fact that the gradient between the arterial and venous blood line declined. Thus, the increased transfer of cooled blood by higher blood pump speeds to the "patient" by far outweighs the effect on the gradient between the arterial and venous blood temperature.

Main drawbacks of the present study are the absence of in-vivo data and the fact that water was used instead of blood, which might have resulted in somewhat different thermal effects. Therefore, despite the fact that in the present study a correction for specific thermal capacity and density of water, ETR values obtained during the present study can, in an absolute sense, not be directly extrapolated to the in-vivo situation. Still, also because blood density and heat capacity of blood are constants, it is likely that relative differences between the various techniques will not be influenced by this factor to a large degree.

More importantly however, in-vivo, the dialysis procedure itself results in changes of core temperature, and therefore in arterial temperature, which cannot be modeled during an in-vitro study. Notably, the effect of contact between blood, dialysis membrane and dialysate, might influence core temperature in vivo through cytokine induction Another pivotal factor in the in-vivo situation is the decline in blood volume. It has been convincingly shown that the ultrafiltration factor during dialysis treatment has profound thermal effects, in the sense that vasoconstriction caused by hypovolemia leads to a reduction in heat loss from the skin and thus, to an tendency towards a rise in core temperature during dialysis[12-14]. This factor explains the strong relation between the decline in blood volume and the extracorporeal energy loss needed to keep core temperature stable during hemodialysis[12]. Moreover, it should be kept in mind that during filtration techniques, progressive hemoconcentration in the membrane can reduce filtration fraction, which might influence ETR in the in-vivo situation.

Thus, although the results of the present in-vitro study obtained during conventional dialysis techniques were fairly comparable with earlier in-vivo data[5,6,9,15], absolute values for ETR obtained during the present study should not be extrapolated to the in-vivo situation. The values obtained in the present study only reflect relative differences in energy transfer which are evoked by changes in dialysate or infusate temperature and heat loss from the blood lines during different extracorporeal blood flow rates and treatment modalities.

Nevertheless, the results of the present in-vitro study obtained during conventional dialysis techniques are concordant with our earlier in-vivo data[5,6,9,10,13].

Concluding, on-line techniques have widely varying effects on ETR during dialysis. The thermal effects of pre-dilution on-line HF with an infusate temperature of 37.5°C appear to be comparable to hemodialysis at 36.5°C whereas post-dilution on-line HDF has thermal effects compared to hemodialysis at 37.5°C. The thermal effect of the blood pump speed was most pronounced during the procedures with the most pronounced energy loss from patient to extracorporeal circuit. The main implications of the present study are the fact that in future clinical studies, comparing conventional dialysis

techniques with on-line procedures, strong attention should be payed towards the thermal effects of the used dialysis procedures. Moreover, when studying the effects of dialysis procedures on hemodynamic stability, also extracorporeal blood pump speed has to be taken into account.

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Chapter 3

Hemodynamics and electrolyte balance: a comparison between on-line pre-dilution hemofiltration and hemodialysis

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Nephrology Dialysis Transplantation 2004;19:2354-9

Abstract

Background
An important advantage of convective therapies is an improved vascular reactivity. However, it is not well known whether the vascular response during convective therapies remains superior when compared to hemodialysis (HD) with an adjusted temperature of the dialysate. It has also been suggested that convective therapies may impair small electrolyte removal through an effect on the Donnan equilibrium. In the present study, we compared the hemodynamic response and small electrolyte removal between pre-dilution on-line hemofiltration (HF) and HD procedures.

Methods
Cardiac output (CO), central blood volume (CBV; liter) and peripheral vascular resistance (PVR) were assessed, using the saline dilution technique, in 12 stable patients during HF and HD with two different temperatures of the dialysate (36.5°C and 35.5°C ($HD^{36.5}$ and $HD^{35.5}$) Balances for sodium, potassium, calcium and conductivity were assessed using total dialysate/filtrate collections. Target filtration volume for HF was 1.2 times body weight. Temperature of the infusate during HF was 36.5°C.

Results
The change (Δ) in CBV was less during HD with a dialysate temperature of 35.5°C (-0.03±0.14 l; $p<0.05$) compared to HF (-0.16±0.05 l) and $HD^{36.5}$ (-0.11±0.14 l), but the other hemodynamic parameters did not differ between the studied techniques. ΔPVR was significantly related to ΔCBV ($r=-0.46$; $p<0.01$), whereas ΔCBV was related to ultrafiltration rate ($r=-0.34$; $p=0.05$). ΔCO was related to ΔCBV ($r=0.62$; $p<0.001$). Solute balances did not differ between HF and HD.

Conclusion
Using the saline dilution method, no difference in the change in CO and PVR was observed between on-line HF versus $HD^{36.5}$ and $HD^{35.5}$. Only CBV declined to a significantly lesser degree during $HD^{35.5}$, although absolute differences were small. Changes in the other hemodynamic variables appeared more dependent upon the degree and rapidity of fluid removal than upon the treatment modality. No difference in small electrolyte balance was observed between HF and HD, suggesting that ionic removal is not impaired during on-line HF.

Introduction

It has long been known that vascular reactivity is better maintained during convective therapies compared with conventional hemodialysis (HD) treatment[1]. Evidence from the early eighties, from the group of Maggiore, showed that temperature-related factors played a major role in the improved vascular response during hemofiltration (HF)[2]. It was also first demonstrated by the Firenze group, and confirmed by others, that significant cooling over the extracorporeal circuit occurred during convective techniques[2,3]. This extracorporeal blood cooling antagonises the increase in core temperature, which is an important cause for the inadequate peripheral vasodilation observed during standard HD therapy.

There is still debate in the literature whether the beneficial hemodynamic effects of convective therapies are primarily due to a cooling effect or to an enhanced removal of vasodepressor substances[4]. HF with on-line production of substitution fluid would appear an ideal model to study the hemodynamic relevance of convective clearance per se, due to the possibility to exchange very large volumes[5]. Studies with detailed hemodynamic measurements during on-line convective therapies are scarce. Moreover, even when the temperature of dialysate and infusate is equal, extracorporeal energy balance may differ between on-line convective techniques and HD[6]. In an in-vitro study, the extracorporeal energy loss during on-line HF with an infusate temperature of 37.5°C appeared to approximate that of HD with a temperature of 36.5°C[7].
However, also changes in electrolyte status may interfere with the cardiovascular response during renal replacement therapies. Whereas sodium balance is mainly of relevance for the preservation of blood volume, calcium and potassium balance may respectively influence the cardiac and vascular response during fluid removal. It is well conceivable that during HF, the increased viscosity in the artificial membrane leads lo a progressive adhesion of negatively loaded proteins, yielding a reduction in the Donnan factor and a reduced mass transport of positively loaded anions[8]. On the other hand, it has also been suggested that increased shear stress by the large infusion volumes during HF may actually reduce the thickness of this protein layer[9]. The convective transport of calcium during pre-dilution HF may also be influenced by dilution of plasma proteins[9].
Few data exist on electrolyte balance during on-line convective therapies, mostly based on calculation of sodium sieving coefficients. However, calculation of electrolyte balance from sieving coefficients may be hazardous during HF, as the protein layer at the surface membrane might increase during progressive hemoconcentration[8]. To our knowledge, electrolyte balance has not yet been compared between HD and on-line HF.

Aim of the present study was firstly to compare the hemodynamic response during pre-dilution on-line HF with HD using different dialysate temperatures and secondly to compare the mass balance of small electrolytes between HD and pre-dilution on-line HF.

Methods

During three different sessions, performed at the same day of the week with weekly intervals, the hemodynamic response was assessed during respectively pre-dilution on-line HF (infusate temperature 36.5°C), and HD using respective dialysate temperatures of 36.5 ($HD^{36.5}$) and 35.5°C ($HD^{35.5}$). Sessions were performed in random order. Electrolyte mass balances were assessed during on-line HF and the HD session with the dialysate temperature of 36.5°C. The patients underwent treatment with standard HD sessions (dialysate sodium 140 mmol/l) in between the two different study sessions.
The target infusate volume during HF was aimed at 1.2 times body weight. Mean blood flow rate during HD sessions was 375±33 ml/min. Treatment time was 4.1±0.3 hours for both HD and HF treatments. Electrolyte composition of the dialysate and infusate was equal: sodium 140 mmol/l, potassium 2 mmol/l, calcium 1.50 mmol/l, glucose 5.6 mmol/l, bicarbonate individualised between 32 and 36 mmol/l. HD and HF were both performed with polyamide membranes (Polyflux® 8L; surface area 1.7m^2 and Polyflux 24S, surface area 2.4m^2, respectively; Gambro; Lund; Sweden). During HD, sterile dialysate was used (<1CFU/liter; endotoxins<0.03 IU/l), using two U-8000S polyamide filters (Gambro; Lund; Sweden). During HF, substitution fluid was sterilized according to the three –filter system (2 U-8000S and 1 disposable U-2000).
Twelve stable patients were included. Exclusion criteria were severe coronary or congestive heart failure (NYHA III or higher) and/or diabetes mellitus. Patient characteristics are summarized in table 3.1. Antihypertensive agents used were angiotensin converting enzyme inhibitors/ angiotensin receptor antagonists (n=7), beta blocking agents (n=7), and calcium antagonists (n=5). Antihypertensive agents were withheld on the day of the study. None of the patients were treated with central venous catheters. The Ethical Committee of the University Hospital Maastricht approved the study. All patients gave written informed consent.
Fluid status was assessed in the patients during an inter-dialytic day by means of echography of the inferior caval vein (criteria for normovolemia 8.5-11.5 mm/m^2) and by assessment of extracellular water by multifrequency bioimpedance analysis (Xitron BIS 4000; San Diego, CA USA).

Table 3.1 Baseline characteristics.

Age (years)	69 ± 6
Sex (M/F)	8 / 4
Weight (kg)	71 ± 11
Body surface area (kg/m^2)	1.82 ± 0.18
Systolic blood pressure (mmHg)	137 ± 17
Diastolic blood pressure (mmHg)	78 ± 10
Number of antihypertensives	1.6 ± 1.0
Inferior caval vein diameter (mm/m^2)	9.6 ± 2.3
Extracellular volume (l/kg)	0.26 ± 0.03
Hemoglobin (mmol/l)	7.6 ± 0.6
Serum albumin (g/l)	36.1 ± 2.7
C-reactive protein (mg/l)	9.2 ± 8.4
Serum sodium (mmol/l)	140.4 ± 2.9
Serum potassium (mmol/l)	5.3 ± 0.6
Serum calcium (mmol/l)	2.35 ± 0.17
Serum phosphate (mmol/l)	1.74 ± 0.41

Hemodynamic measurements

Cardiac output (CO), central blood volume (CBV) and peripheral vascular resistance (PVR) were assessed by the saline dilution technique (Transonic HD 01®; Transonic Systems; Ithaca NY; USA) as described in detail elsewhere[11]. In short: a heated (37°C) bolus of 30 ml NaCl 0.9% (indicator) is injected into the venous line with the blood pump speed set at 200 ml/min, and the change in velocity of ultrasound waves produced by the returning dilution curve (S) is detected by a probe attached to the arterial line. By comparing the dilution curve with a calibration curve (S_{cal}), produced by injecting 10 ml of isotonic saline in the venous bubble trap, CO is calculated by the formula:
3 * *blood flow* * (S / S_{cal}). CBV, which is considered to be the blood in the heart, great vessels (pulmonary artery and veins and escending aorta) and the lung capillaries, is calculated by multiplication of CO with the mean transit time of the indicator, corrected for travel time in the arterial and venous blood line. PVR is calculated by dividing mean arterial pressure by CO.
Measurements were performed immediately after the start and at the end of the treatment, and in the middle of the dialysis session.
Measurements were performed in duplicate and the mean value was used in the analysis. Coefficient of variation between the duplicate measurements was 8.3% for CO, 8.8% for CBV and 9.1% for PVR. Access recirculation was 0% in all patients.
The change in relative blood volume (RBV) was assessed with the blood volume sensor (BVS) system (Gambro; Lund Sweden). Blood pressure was measured manually (Speidel and Keller; Maxi Stabil 3; Jungingen; Germany).
Body temperature was measured with an ear thermometer (Genius® First Temp Model 3000A, St.Louis, USA).

Calculation of solute balance

Solute balances (mmol/session) during HD were calculated according to the following formula: $[V_{out} \times C_{out}] - [V_{in} \times C_{in}]$, in which V_{out} = volume of spent dialysate, C_{out} = concentration of solute in spent dialysate, V_{in} = volume of fresh dialysate, C_{in} = concentration of solute in dialysate[8]. During HF, basically the formula for solute mass balance is $[Vf_{out} \times Cf_{out}] - [Vf_{in} \times Cf_{in}]$[8]. Vf_{out} and Cf_{out} are respectively the filtrated volume and the concentration of the solute in the filtrate. Vf_{in} and Cf_{in} are the infused volume and the concentration of the solute in the infusate. During on-line HF, mixing purified water with concentrates produces infusate. However, not all of the fluid which is produced in this way is infused in the patient, and the unused fluid is directly removed through the bypass of the module, entering the collection box through the same port as the filtrate which is removed from the patient. Thus, the collection box contains a mixture of filtrate and unused fluid and a correction factor has to be applied. The filtrate can be calculated as the sum of infusate and ultrafiltration volume, and the unused fluid as the product of the "total fluid" flow rate [which is read as dialysate flow on the monitor] and dialysis time. Thus, a ratio (R) between filtrate volume and unused fluid can be calculated as follows: filtrate volume divided by ("total fluid" "flow rate" x dialysis time). In this way, the corrected concentration of the solute can be calculated as follows: $Cf_{out} = ((Cf_{collection\ box} - (R^* Cf_{in}))/(1-R)$. Cf_{out} can than be used in the basic formula for solute mass balance for hemofiltration.

Alternatively, the amount of solutes removed from the patient during HF can be calculated according to the formula: $[Vf_{in} - Cf_{in}] - [Vf_{out} \times Cf_{out} - R \times (Vf_{in} \times Cf_{in})]$, which yielded appoximately the same results as the previous approach.

We also used conductivity balance in addition to sodium mass balance. Dialysate conductivity is mainly determined by dialysate sodium[12]. We included a secondary method for the assessment of sodium balance, as due to the inherent variability of electrolyte measurements and the large volumes, calculation of electrolyte mass balances during dialysis may suffer from some inaccuracy.

Electrolyte concentrations were assessed by indirect ionometry (Synchron LX 20; Beckman® Coulter; Brea; CA; USA). In contrast to direct ionometry, indirect ionometry does not need a correction factor when assessing sodium in aqueous media and yields results comparable to flame photometry[13]. Two samples were taken and the mean of the two used for calculations. The coefficient of variation for sodium measurements in dialysate in the present study was 0.7%.

Statistics

Values are expressed as mean ±SD. Differences within and between the three treatment modalities were assessed using repeated measurements ANOVA. If significant result were further analyzed using paired and unpaired Student t-test. Multiregression analysis was also used to assess the relation between the treatment modality and hemodynamic changes. Correlation between hemodynamic variables were assessed using pearson's r.

Results

In one patient, transmembranous pressure (TMP) immediately increased to maximal levels during HF, and the treatment had to be terminated. Thus, the study was completed in 11 patients. Ultrafiltration volume was comparable between the three treatment sessions (HF:2.4±1.0 l; HD (36.5°C): 2.4±0.8; HD (35.5°C):2.7±0.8 l). Mean filtration volume achieved during HF was 75±9 l.
RBV decreased significantly during HF (-9.7±3.1%), $HD^{36.5}$ (-8.0±3.4%) and $HD^{35.5}$ (-7.7±4.0%) [p<0.001]. The change in body temperature did not differ between HF (0.1±0.6°C), $HD^{36.5}$ (0.3±0.6°C) and $HD^{35.5}$ (0.0±0.4°C). Systolic and diastolic blood pressure (BP) did not change significantly during either HF (-1.6±19.8 and –7.1±9.9 mmHg), $HD^{36.5}$ [-0.8±22.7 and –3.8±12.4 mmHg] and $HD^{35.5}$ (-6.0±22.5 and –4.1±7.6 mmHg).
Hemodynamic data obtained by the saline dilution technique are summarized in table 3.2.
During HF and $HD^{36.5}$ but not during $HD^{35.5}$, CBV declined significantly (p<0.05 compared to the other treatment modalities), whereas the decline in CO only reached significance during HF. ΔPVR and ΔCO did not differ between the various treatment modalities. Pooling all treatments, the changes (Δ) in CO and ΔCBV were significantly and positively related (r=0.62; p<0.001), whereas ΔCBV and ΔPVR were inversely related (r=-0.46; p<0.01). ΔCO was significantly related to ΔSystolic BP (r=0.36;p<0.05) and ΔDiastolic BP (r=0.53; p<0.001). Multiregression analysis only identified treatment modality as an independent predictor for ΔCBV, but not for ΔPVR or ΔCO.

Table 3.2 Hemodynamic measurements during the different treatment modalities.

	HF	$HD^{36.5}$	$HD^{35.5}$
CO start	6.9 ± 1.3	6.5 ± 1.1	6.2 ± 1.2
CO mid	6.4 ± 1.1	6.3 ± 1.2	6.3 ± 1.4
CO end	6.0 ± 1.2*	6.1 ± 1.3	5.7 ± 1.0
ΔCO	-0.8 ± 1.0	-0.4 ± 1.0	-0.5 ± 0.9
CBV start	1.43 ± 0.34	1.37 ± 0.33	1.27 ± 0.33
CBV end	1.27 ± 0.30*	1.27 ± 0.30*	1.24 ± 0.30
ΔCBV	-0.16 ± 0.05	-0.11 ± 0.14	-0.03 ± 0.14
PVR start	15.2 ± 3.6	14.7 ± 2.1	17.3 ± 5.4
PVR mid	15.8 ± 2.8	15.1 ± 2.6	16.6 ± 3.2
PVR end	16.4 ± 4.1	15.5 ± 3.0	17.5 ± 4.1
ΔPVR	1.2 ± 2.3	0.8 ± 1.5	0.2 ± 3.4

* $p<0.05$ compared to start of treatment; #compared to the other treatment modalities; HF=hemofiltration; $HD^{36.5}$=hemodialysis with dialysate temperature of 36.5°C; $HD^{35.5}$=hemodialysis with dialysate temperature of 35.5°C; CO=cardiac output (l/min); CBV=central blood volume (l); PVR=peripheral vascular resistance (mmHg/min/l); Δ=change during treatment (end versus start); mid=middle of dialysis treatment.

When corrected for weight loss, ΔRBV was related to both ΔCO (r=-0.56; $p<0.001$) and ΔCBV (r=-0.34; p=0.05). Ultrafiltration rate was related to ΔRBV:weight loss (r=0.43; $p<0.001$), Ultrafiltration rate was also related to ΔCBV (r=-0.34; p=0.05) and ΔCO (r=-0.59; $p<0.001$).

Regarding the balance studies, data are presented in table 3.3. The balance for sodium, potassium, calcium and phosphate did not differ between HF and HD treatments. Also conductivity balance did not differ between HF and HD. Sodium concentration of infusate and fresh dialysis fluid was respectively 139.7±0.8 and 140.0±2.0 mmol/l. The mean sodium concentration in spent dialysate (140.8±2.6 mmol/l) and filtrate (140.6±2.2 mmol/l) (applying the correction factor for the unused fluid) was comparable. With HF, the correlation between sodium balance and ultrafiltration volume was highly significant (r=-0.93;$p<0.001$). With HD, this relation was less strong, but still significant (r=-0.61; $p<0.05$).

Urea reduction ratio was 61±5% for HF and 71±6% for HD ($p<0.05$).

The change in serum sodium (-0.4±3.0 versus -1.0±2.8 mmol/l), potassium (-1.6±0.3 versus -1.6±0.4 mmol/l), calcium (0.02±0.2 versus –0.08±0.2 mmol/l), and phosphate (0.9±0.2 versus –0.9±0.4 mmol/l) did not differ between HF and HD.

Table 3.3 Solute balance during HF and HD.

	HF	HD
Sodium (mmol/treatment)	-436 ± 278	-365 ± 233
Potassium (mmol/treatment)	-92 ± 28	-88 ± 22
Calcium (mmol/treatment)	-4.6 ± 7.1	-4.8 ± 6.5
Phosphate (mmol/treatment)	-30.4 ± 6.0	-29.2 ± 9.0
Conductivity (mS/cm/treatment)	-61.5 ± 12.4	-52.4 ± 8.8

Discussion

In the present study, no major differences in the change in CO or PVR, were observed between on-line predilution HF (infusate temperature 36.5°C) and HD with dialysate temperatures of respectively 36.5°C and 35.5°C. CBV was slightly, but significantly better maintained during HD[35.5] compared to the other techniques.

Earlier studies showed convincingly a superior hemodynamic stability during pre-dilution on-line HF when compared with HD[4]. However, although the temperature of the dialysate and infusion fluid during on-line therapies were matched (37.0°C) in these studies, in-vivo and in-vitro data showed that pre-dilution on-line HF exerts a more pronounced cooling effect compared to HD even if the temperature of dialysate and infusate are equal[3,7].

In the present study, PVR was studied using the saline dilution technique. The saline dilution technique was recently introduced in dialysis therapy and yielded promising results in the assessment of the hemodynamic response during dialysis[13-15]. In the present study and in reports by others, the change in CO, which is independently measured by the saline dilution technique, was significantly related to changes in blood pressure. However, we were puzzled by the fact that, despite the fall in CO, PVR did not increase significantly during the treatment sessions. Also, in a study by Hoeben, no differences in PVR were observed between cool dialysis and standard dialysis[14], whereas in the report by Prakash, no increase in PVR was observed after ultrafiltration[15]. The absence of a change in PVR during on-line HF is in contrast with earlier studies of our group using strain gauge plethysmography[3]. The reason for this discrepancy is not clear. Dilution techniques to measure PVR are well established in the intensive care unit. However, it should be kept in mind that in this setting, mean arterial pressure is usually assessed by direct intra-arterial monitoring, and not by indirect measurements like in the present study. Also, in contrast to measurements by dilution techniques, strain gauge plethysmography mainly assesses the reactivity of the forearm skin blood vessels, which are highly sensitive to thermal changes. In a recent study in 13 hemodialysis patients, we observed a difference between the cutaneous vascular response

assessed with laser doppler flowmetry, and the systemic vascular response studied by the saline dilution technique. The treatments compared in this study were so-called thermoneutral HD (in which extracorporeal energy transfer is zero but core temperature increases) and isothermic HD (in which core temperature is maintained stable by removing energy from the extracorporeal circuit). Despite significant differences in skin blood flow (which decreased more during thermoneutral dialysis compared to energyneutral dialysis; -0.93±0.6 AU versus –0.45±0.9 arbitrary units; $p<0.05$)[16], the change in peripheral vascular resistance assessed by the saline dilution technique was comparable (5.5±5.1 versus 6.0±8.5 mmHg/l/min; p=ns). Therefore, it appears likely that small differences in cutaneous vascular reactivity may not be detected by the saline dilution method.

ΔPVR was significantly related to ΔCBV, which was in turn related to ultrafiltration rate and ΔRBV. Thus in the present study, the change in PVR measured by the saline dilution technique appeared to be more dependent upon the degree and rapidity of fluid removal than upon the treatment modality used. However, as discussed previously, local differences in vascular reactivity may be missed by the saline dilution technique.

Although the lack of a change in PVR might also theoretically be explained by relative overhydration of our patients, the normalized extracellular volume, assessed by bioimpedance analysis, was comparable to reported values of dialysis patients in an international study and to values observed in stable renal transplant patients[17,18].

Though absolute differences were small, the fall in CBV was lower during $HD^{35.5}$ compared to the other techniques, especially during the first half of the dialysis treatment. In the study of Hoeben[14], also CBV was more sensitive than PVR in detecting hemodynamic changes between different techniques. The reason for the lesser fall in CBV during $HD^{35.5}$ compared to the HF treatment could be that extracorporeal blood cooling may still have been somewhat larger during the former treatment, resulting in improved peripheral venoconstriction and mobilisation of peripheral blood volume to CBV. More detailed thermal studies should shed more light on this item. However, the observed differences were small and of uncertain hemodynamic significance. Indeed, also during HF and during $HD^{36.5}$, the fall in CBV was small (±100 ml) despite a mean ultrafiltration volume of 2500 ml and the significant fall in RBV. This might be an argument for adequate mobilisation of fluid volume from the peripheral to the central compartments also during these treatment modalities. Thus, an adequate mobilisation of unstressed blood volume, resulting in only a minor decline in CO, may not necessitate a large increase in PVR in order to maintain blood pressure.

Apart from possible limitations of the hemodynamic measurements, other potential drawbacks of the present study are firstly the inclusion of stable

patients. This was done because the scope of this study was to assess the cardiovascular response during different treatment modalities and not the frequency of intra-dialytic hypotensive episodes per se, which would have necessitated a different study design. However, differences in vascular reactivity between different dialysis techniques are not limited to unstable dialysis patients, but can be very well studied in stable patients, as shown in earlier studies by others and by our group[1-3,19]. Secondly, during the different techniques, fluid was removed with relatively modest ultrafiltration rates until dry weight. Hemodynamic differences might be more pronounced if more agressive ultrafiltration would have been applied. However, in previous studies were shown differences in the blood pressure response between different dialysis modalities using the same approach[19]. Moreover, the number of patients included was relatively small, although we and others[1-3,14] have shown profound hemodynamic differences between various dialysis modalities using approximately the same number of patients. In the present study, ear thermometry was performed in order to investigate changes in core temperature, which may have yielded less precise results compared with earlier studies, in which core temperature was measured by direct blood temperature monitoring. However, for technical reasons this was not possible in the present study.

No differences in sodium, potassium and calcium balances were observed between on-line HF and HD, though, due to the inherent variability of the electrolyte measurements and, especially the high standard deviation of the sodium measurements, the results should be interpreted with caution. However, also no difference in conductivity balance was observed between the two techniques. Moreover, sodium concentration in spent dialysate and filtrate were nearly equal, whereas also plasma sodium concentrations did not change during HF treatment. All these factors argue against hypotonic fluid removal during pre-dilution on-line HF.

Interestingly, we were able to calculate sodium mass balance during HF, yielded results that were highly significantly related to ultrafiltration volume, which is a necessity for the reliability of sodium balance measurements. This strong relation with ultrafiltration volume explains the high standard deviation of sodium mass balance measurements. However, the relation between ultrafiltration volume and sodium balance during HD was, although significant, far less strong. Thus, especially during HD, the results should be interpreted with caution. The reason for the lesser reliability of sodium balance measurements during HD is not clear but might be related to the higher volumes used in the formula for dialysate, augmenting errors due to the variation of the sodium measurements.

Our findings are in some contrast with an earlier study showing hypotonic fluid removal by pre-dilution HF[8]. On the other hand, David et al. showed an

reduction in sodium sieving during pre-dilution compared to post-dilution HF[10]. Locatelli also observed a reduction in the Donnan factor (α) using pre-dilution HF[8]. In the present study, we used far higher infusion volumes than in the latter study, which because of the large dilution, is likely to have resulted in a lesser effect on α.

We chose to perform balance studies by the collection method instead of studies on electrolyte sieving, as the latter may change during the filtration treatment due to progressive protein adhesion to the artificial membrane. Indeed, a progressive rise of TMP, especially during the last hour of the HF treatment, may be a limiting factor in the prescription of the quantity of the filtration volume. This occurred in some of our patients and explains that the filtration volume achieved during HF on-line was in individual cases, less than the prescribed volume.

Though not of direct relevance for the hemodynamic response, phosphate removal was also assessed, which was also not different between HD and HF. These results are in agreement with an earlier studying comparing phosphate removal between conventional HF and HD[20]. Despite the fact that phosphate clearance is expected to be higher with the use of the larger and more permeable membrane during HF, phosphate removal might be influenced by restrictions in phosphate transfer from different body compartments to the intravascular compartment[20] and by the limitations in the filtration volume.

Summarising, using the saline dilution method, no difference in the change in CO and PVR was observed between on-line HF and $HD^{36.5}$ and $HD^{35.5}$. Only CBV declined to a slightly, but significantly lesser degree during $HD^{35.5}$.

In contrast, the change in PVR during the different dialysis strategies was strongly and inversely related to the change in CBV, which was in turn related to ultrafiltration rate and the change in RBV. Thus, the change in PVR, as measured by the saline dilution technique, appeared more dependent upon the degree and rapidity of fluid removal than upon the treatment modality. However, results should be interpreted with caution, as the saline dilution technique may fail to detect local differences in vascular tone. No difference in small electrolyte balance was observed between HF and HD, suggesting that ionic removal is not impaired during pre-dilution on-line HF.

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Chapter 4

Nitric oxide synthetic capacity in relation to dialysate temperature

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Blood Purification 2004;22:203-9

Abstract

Background

During hemodialysis, vascular reactivity is impaired, which can be corrected by lowering dialysate temperature. It has also been shown that nitric oxide (NO) is related to intra-dialytic hypotension. As NO synthesis may be temperature dependent, this study addressed the influence of dialysate temperature on the NO synthetic capacity of plasma.

Methods

NO synthetic capacity were studied during hemodialysis with a dialysate temperature of 37.5°C (dialysis-37.5°C) and programmed extra corporeal blood cooling (cool dialysis; Blood Temperature Monitor; Fresenius®) in 12 stable patients. NO synthetic capacity was assessed ex vivo by [3H] L-citrulline formation from [3H] L-arginine in cultured endothelial cells after incubation with plasma samples obtained during the respective sessions.

Results

Core temperature decreased (-0.32±0.10°C) and energy transfer rate was significantly lower (-27.5±2.8 W; $p<0.05$) during cool dialysis compared to dialysis-37.5°C (0.19±0.06°C and -0.8±1.2 W respectively; $p<0.05$). Systolic blood pressure decreased during dialysis-37.5°C (-19±4 mmHg; $p<0.05$), but not during cool dialysis (-6±5 mmHg).

NO synthetic capacity increased during dialysis-37.5°C (55.5±9.3->73.5±10.2 pmol/105 cells; $p<0.05$), in contrast to cool dialysis (67.3±11.1->66.2±10.8 pmol/105 cells).

Conclusion

The stimulatory effect of uremic plasma on endothelial NO synthesis was augmented during dialysis-37.5°C but not during cool dialysis.

Introduction

Next to the decline in blood volume, an inadequate constriction of both resistance and capacitance vessels during hypovolemia is an important contributory factor to intradialytic hypotension.[1] From the literature, two main explanations for this phenomenon have emerged. The first is the "heat stress" hypothesis, which proposes that the increase in core temperature, which is commonly observed during hemodialysis (HD) with dialysate temperatures of 37-37.5ºC, leads to cutaneous vasodilatation in order to remove the excess heat.[2,3,4] This phenomenon, which is in accordance with studies in patients with heat stroke[5], antagonizes the physiologic response to hypovolemia.[6-8] Evidence for this hypothesis resides in the observations that vascular reactivity is improved with the use of so-called "cool" HD (dialysate temperature 35-36ºC), during isolated ultrafiltration and during convective renal replacement techniques.[6-9] All the latter procedures are associated with a significant energy loss over the extra corporeal circuit.[4,7,9,10] The second explanation for the impaired vascular response during hemodialysis is the "nitric oxide" hypothesis, which proposes that vascular reactivity is impaired by an elevation of nitric oxide (NO) synthesis, a potent vasodilatator, during the dialysis treatment.[11-14] Evidence for this hypothesis resides in the fact that NO levels increase during HD and appear to be higher in those patients experiencing hypotensive episodes during HD.[15-17] Moreover, inhibitors of NO synthesis had a preventive effect on the occurrence of hypotensive episodes.[18] The cause of the increase in NO synthesis is not yet clear. It has been suggested that this phenomenon may be mediated by stimulation of monocytes through contact of blood with the artificial membrane or the dialysis fluid, or by removal of endogenous nitric oxide inhibitors, or by endothelial activation.[15,19,20,21]

However, data from experimental studies suggest that NO metabolism may also be temperature dependent. In an in-vitro study, inducible NO synthase II activity was stimulated by an increase in temperature.[22] Therefore, we hypothesized that the dialysate temperature during HD might influence NO synthesis, which would result in a reduction in NO generation during cool dialysis compared with HD with a dialysate temperature of 37.5°C (dialysis-37.5°C).

The aim of the present study was to compare the effects of dialysis-37.5°C and cool dialysis on the NO synthetic capacity of plasma obtained during these respective treatment modalities.

Patients and methods

Patients

Twelve stable patients were selected from the chronic HD population from the University Hospital of Maastricht. Patient characteristics are summarized in table 4.1. Patients were dialyzed three times a week, except one patient who was dialyzed for two times a week. Original renal disease was chronic glomerulonephritis in 4 patients, polycystic kidney disease in 3 patients, bilateral kidney removal due to renal cell carcinoma in one patient, nephrosclerosis in 2 patients, and chronic pyelonephritis in 2 patients. Seven patients were on antihypertensive agents, which were withheld on the day of the investigation. Two patients were smokers. All patients were treated with subcutaneous erythropoietin and intravenous iron. Intravenous iron therapy and vitamin supplements were withheld from two weeks before the start of the study, to avoid a possible interference with NO metabolism.

Table 4.1. Patient characteristics.

n	12
Age (years)	64.7 ± 2.6
Sex (M/F)	7 / 5
Urea reduction rate (%)	70.5 ± 1.7
C-reactive protein (mg/l)	7.3 ± 2.1
Dry weight (kg)	75.6 ± 4.0
Time on dialysis treatment (months)	26.6 ± 5.8
Duration of dialysis session (minutes)	230 ± 5.8
Hemoglobin (mmol/l)	7.2 ± 0.2
Serum ferritin (µg/l)	265 ± 61
Erythropoietin dose (IU/week)	7000 ± 674
Intravenous iron dose (mg/month)	200 ± 22

Data as mean±SEM.

Patients with diabetes mellitus, severe cardiac disease (left ventricular ejection fraction below 25%, coronary heart disease or heart failure NYHA III or IV), or those using nitrates were excluded from the study. For ethical reasons, only stable dialysis patients were included, as a dialysate temperature of 37.5°C is expected to result in peripheral vasodilatation and therefore to an enhancement of intra-dialytic hypotension.

All patients gave informed written consent for the study. The Ethical Committee of the University Hospital Maastricht approved the study.

Study design

NO synthetic capacity was assessed in plasma obtained during two different dialysis treatments in 12 HD patients. The first treatment was bicarbonate HD with a dialysate temperature of 37.5°C (to be called "dialysis-37.5°C"), and the second treatment was HD with programmed extra corporeal blood cooling (to be called "cool dialysis"). Energy transfer rate during cool dialysis was set at an equivalent rate as was measured during a session of isolated ultrafiltration in the same patient. This approach was chosen because earlier studies have shown that differences in vascular reactivity are maximized when dialysis-37.5°C and isolated ultrafiltration or HD with an extra corporeal energy transfer equal to isolated ultrafiltration were compared.[7,8,23] The session with isolated ultrafiltration was performed one week before the start of the study.
In order to prevent influences of different volume status, the same day of the week was chosen for both treatment sessions, which were performed in random order with an interval of one week. In all treatments ultra pure water [achieved by filtration of reverse-osmosis treated water by polysulfone (Diasafe®; Fresenius)] and synthetic dialysis membranes (polyamide; Polyflux 8-L®; Gambro, Sweden) were used. Blood flow rate was set at 300 ml/min and dialysate flow at 500 ml/min. Dialysate sodium concentration was 140 mmol/l and dialysate calcium concentration 1.50 mmol/l, bicarbonate was individualized (32-36 mmol/l) and dialysate glucose concentration was 1 g/dl. Anticoagulation was performed with low molecular weight heparin. No food or beverages were allowed during the dialysis session.

NO synthetic capacity

NO synthetic capacity was assessed ex vivo by measuring the stimulatory effect of uremic plasma on endothelial NO synthesis. Just before the start of the dialysis session, after 5, 15 and 60 minutes, and at the end of the dialysis sessions blood was drawn from the arterial line and placed on ice immediately. The blood samples were centrifuged for 15 minutes at 4000 rpm at a temperature of 4°C. All the plasma samples were frozen and stored at -80°C immediately until later analysis. NO synthetic capacity was assessed by [^{3}H]-L citrulline formation from [^{3}H]-L arginine by human umbilical vein endothelial cells (HUVEC), after incubation with the respective plasma samples.[5,15] HUVECs were washed twice with phosphate buffer saline (PBS) and incubated for 24 hours with 1 mL of heparinized plasma (diluted 1:2 with PBS) containing 0.5 µCi [^{3}H]L-arginine (New England Nuclear, Boston, MA, USA; 56.4 Ci/mmol). One-milliliter aliquots of plasma (diluted 1:2) from each subject, containing [^{3}H]L-arginine, were incubated without cells and used as blanks. Incubation was stopped by adding one volume of ice-cold 15% trichloroacetic acid (TCA) to cell supernatants and NO generation was evaluated by

measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline.[15] Trichloroacetic acid-treated samples were centrifuged at 10,000 g to precipitate proteins. Thereafter, supernatant were extracted with ether, vacuum lyophilized, resuspended in 2 mL HEPES, pH 5.5, and applied to 2 mL wet bed volumes of Dowex AG 50 WX-8 (100 to 200 mesh, Li^+ form, Bio-Rad, Richmond, CA, USA) followed by 2 mL of water. [^{3}H]L-citrulline were quantitated in the column effluent by liquid scintillation counting and identified as described.[15] Results were expressed as pmoles/10^5 HUVEC by correcting data in counts per minute for the specific activity of [^{3}H]L-arginine, calculated on the basis of plasma endogenous content of L-arginine, as measured by high-performance liquid chromatography (HPLC) on aliquots of each plasma sample. The intra-assay variability of this assay is 7%, the inter-assays variability is 10%.

Energy transfer rate

Extra corporeal energy transfer rate was measured and modeled by the Blood Temperature Monitoring (BTM®); Fresenius Medical Care; Bad Homburg; Germany). The BTM® measures temperature at the arterial (T_{art}) and venous line (T_{ven}) of the extra corporeal system with a platinum sensor around the arterial and venous blood lines[5,8] at 15-second intervals. Energy transfer rate is calculated by the BTM® using the following formula: $c \times \rho \times Qb \times (T_{ven}-T_{art})$, with c=specific thermal capacity (3.64 kJ/kg x ^{0}C), Qb=extra corporeal blood flow rate (ml/min), ρ=density of blood (1052 kg/m^3).[8] Values were converted to Watts (1W=3.6 kJ).

Modeling of energy transfer rate by BTM® is achieved by the thermal flux option of the BTM®, which measures T_{art}, T_{ven} and Qb in 15-s intervals and which actually calculates the actual energy transfer rate according to the above mentioned equation. The algorithm of the BTM® uses the information of the actual energy transfer rate by automatically setting and continuously adjusting dialysate temperature in order to reach and maintain the target energy transfer rate.

Core temperature

The core temperature was measured by using the BTM® described above. The BTM® measures the temperature at the arterial side of the fistula and calculates central venous blood temperature by correcting for fistula and cardiopulmonary recirculation. Due to the recirculation test, the first recording of core temperature takes place circa 5 minutes after the start of dialysis. Due to the possible interfering effects of the recirculation tests by BTM® on thermal parameters, core temperature was measured at the start, after 60 minutes, and after 240 minutes.

Hemodynamics

The systolic and diastolic blood pressure was measured by an oscillometric method at the arm contra lateral of the fistula. The device used is incorporated in the Fresenius® 4000H; the device is calibrated at a regular interval. At each point the blood pressure was measured two times, the mean of the two measurements was taken for further analysis. The relative decline in blood volume was measured by an on-line Blood Volume Monitor (BVM; Fresenius®), which measures relative changes in ultrasound propagation by red blood cells during the dialysis session.

Statistical Analysis

Data were analyzed by repeated measurements ANOVA to analyze overall differences throughout and between treatments. If ANOVA was significant, differences in individual points was assessed by the paired Student t-test. Correlation analyses were performed with Student t-test. Statistical analysis was performed using the SPSS-PC statistical package. Data are expressed as mean±SEM. Values below 0.05 were considered significant.

Results

NO synthetic capacity

The stimulatory effect of uremic plasma on endothelial NO synthesis increased significantly during dialysis-37.5°C ($p<0.05$; repeated measurements ANOVA), *both when expressed as relative* (percentage of initial values) or absolute values. Compared to the start of dialysis, NO synthetic capacity reached a significantly different value at 60 and 240 minutes ($p<0.01$ and $p<0.05$; respectively) (figure 4.1 and table 4.2). During cool dialysis, no significant differences were observed between the various points in time. NO synthetic capacity tended to be lower during cool dialysis compared to at 60 minutes compared to the same time point during dialysis-37.5°C, but the difference did not reach statistical significance ($p=0.07$ for relative values). Arginine levels decreased significantly ($p<0.05$) during both hemodialysis sessions (table 4.2).

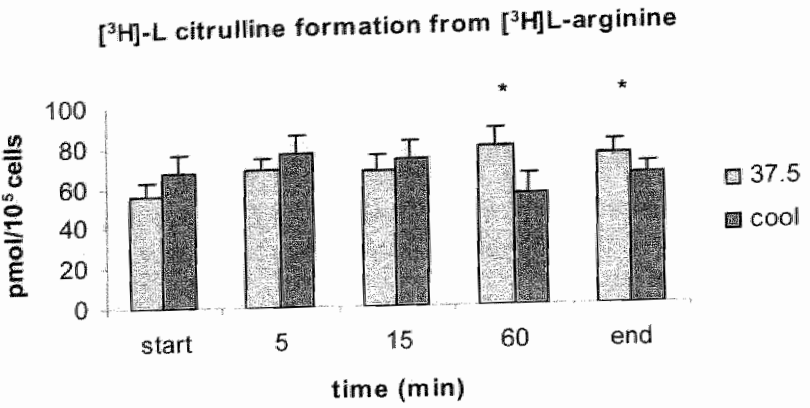

Figure 4.1 NO synthetic capacity during dialysis-37.5°C and cool dialysis
Data presented as mean±SEM. *Values during dialysis-37.5°C obtained at the start of dialysis differed significantly with values obtained at 60 minutes or at the end of dialysis.

Table 4.2 Study parameters.

	Start	*5*	*15*	*60*	*End*
NO synthetic capacity (pmol/105 cells)					
37.5°C	55.5 ± 9.3†	68.4 ± 15.4	66.9 ± 11.8	78.6 ± 14.4*	73.5 ± 10.2*
Cool	71.7 ± 11.1	76.8 ± 11.4	73.2 ± 13.5	55.7 ± 8.7	66.2 ± 10.8
NO synthetic capacity *(% of initial values)*					
37.5°C	100†	124 ± 15	124 ± 18	140 ± 10*	140 ± 16*
Cool	100†	129 ± 22	117 ± 18	96 ± 14	107 ± 16
Arginine *(µmol/l)*					
37.5°C	92 ± 7†	84 ± 6	83 ± 8	81 ± 10	71 ± 7*
Cool	110 ± 9†	88 ± 9†	81 ± 9†	78 ± 10	68 ± 6*
Systolic blood pressure *(mmHg)*					
37.5°C	150 ± 5		151 ± 4	150 ± 4	132 ± 4*
Cool	146 ± 5		145 ± 5	148 ± 5	140 ± 6
Relative blood volume *(%)*					
37.5°C			96.3 ± 0.5**†	93.1 ± 0.9*†	88.4 ± 2.0**
Cool			96.2 ± 0.5**†	91.6 ± 1.1*†	85.4 ± 1.8**
Core temperature (°C)					
37.5°C		36.69 ± 0.06†		36.70 ± 0.07†	36.88 ± 0.11**
Cool		36.48 ± 0.08**†		36.21 ± 0.07	36.16 ± 0.10
Temperature in venous line *(°C)*					
37.5°C		36.64 ± 0.05		36.67 ± 0.06	36.62 ± 0.09
Cool		35.03 ± 0.10**†		34.55 ± 0.09	34.46 ± 0.12

Cool = dialysis with programmed extracorporeal blood cooling'; 37.5°C = hemodialysis with dialysate temperature of 37.5°C.
Values as mean±SEM. NO synthesis expressed by [3H]-L citrulline formation from [3H]-L arginine
*p<0.05 compared to start of dialysis; **p<0.05 compared to 60 minutes after the start of dialysis; †p<0.05 compared to end of dialysis.

Hemodynamics

Systolic blood pressure decreased significantly during dialysis-37.5°C, but not during cool dialysis (table 4.2). Diastolic blood pressure did neither decline significantly during dialysis-37.5°C (81±2 mmHg before dialysis; 76±3 mmHg after dialysis), nor during cool dialysis (81±3 mmHg before dialysis; 79±4 mmHg after dialysis). Changes in blood pressure during both treatments were not significantly related to NO synthetic capacity. Heart rate tended to increase during dialysis-37.5°C (71±2 bpm before dialysis; 82±6 bpm after dialysis; p=0.06), but not during cool dialysis (71±2 bpm before dialysis; 70±3 bpm after dialysis).
Total ultrafiltration volume was comparable between dialysis-37.5°C and cool dialysis (1.8±0.3 l and 1.7±0.2 l, respectively), as was the relative percentage in blood volume (table 4.2). No patient experienced "cold" or "hot" sensations during the study and no hypotensive episodes occurred during the study.

Thermal parameters

Core temperature increased significantly during dialysis-37.5°C and decreased during cool dialysis. The difference in core temperature between cool dialysis and dialysis-37.5°C was significantly different after 60 minutes and at the end of dialysis (table 4.2).
Temperature in the venous line of the extra corporeal circuit remained stable during dialysis-37.5°C and decreased during cool dialysis (table 4.2). Temperature in the venous line was significantly different between cool dialysis and dialysis-37.5°C at all points in time.
Core temperature and temperature in the venous line were not related to NO synthetic capacity. There were no changes in thermal parameters related to changes in NO synthetic capacity during either dialysis-37.5°C or cool dialysis.
Extra corporeal energy transfer rate was significantly lower during cool (-27.5±2.8 W) compared to dialysis-37.5°C (-0.8±1.2 W; $p<0.05$). Mean dialysate temperature during the cool dialysis session was 35.2±0.2°C.

Discussion

The main finding of the present study was the increase in NO synthetic capacity during treatment with dialysis-37.5°C, whereas NO synthetic capacity did not change significantly during cool dialysis. The fact that NO synthetic capacity also tended to increase during the first 5 minutes of cool dialysis could be explained by the fact that the BTM® device does not start to model energy transfer rate before 5 minutes after the start of dialysis. Thus, at the first

measurements, no effect of dialysate temperature on NO synthetic capacity can be expected yet.

The cause of the increase in NO synthetic capacity during dialysis-37.5°C is as yet unknown. It has been hypothesized that induction of inducible NO synthase, which is already up regulated by repeated stimulation of monocytes by the dialysis treatment, is primarily responsible for an increase in NO synthesis during dialysis. Moreover, a removal of endogenous inhibitors of NO, like asymmetric dimethyl arginine (L-ADMA) during hemodialysis, has also been causally implicated.[13,14,16,20,24] On the other hand, the rapid increase in NO generation immediately after hemodialysis has been attributed to a stimulation of the constitutive form of NO synthase by activated platelets or white blood cells.[21] Recently, data obtained in a rat model showed that pump perfusion in an experimental extra corporeal circuit caused endothelial NO release, which was triggered by serotonin from activated platelets.[25] Also, turbulence at the initiation of dialysis or heparin might influence NO release.[26] However, the present study showed an effect of dialysate temperature on NO synthetic capacity, and especially inducible NO synthase appears to be temperature dependent.[22] An explanation for this apparent paradox might be that activation of constitutive NO synthase leads to a rapid increase in NO synthesis. During dialysis-37.5°C, the increase in NO synthetic capacity might be maintained by up regulation of inducible NO synthase by the cytokines released by circulating monocytes, in combination with a diffusive removal of NO inhibitors, or by the increase in core temperature per se.[5] From the present study, it cannot be elucidated whether the small increase in core temperature contributes to an increase in NO synthetic capacity during dialysis-37.5°C, or that the increase in NO synthetic capacity induced by the dialysis treatment itself is abolished by extra corporeal blood cooling during cool dialysis.

Also, it is difficult to elucidate whether the differences in NO synthetic capacity of plasma observed during the two treatment modalities are mainly due to a local or systemic temperature effect. A local effect in the extra corporeal circuit cannot be excluded, certainly in view of the fact that temperature differences in the venous side of the extra corporeal circuit between cool dialysis and dialysis -37.5°C were much more pronounced compared to the differences in core temperature. Nevertheless, these local differences might contribute to a systemic effect on NO synthetic capacity, as blood was drawn from a port in the arterial line (thus: at a point where the cooling effect of the extra corporeal circuit is only minor). Changes in NO synthetic capacity were not directly related to thermal parameters, which might be explained by the fact that, as discussed previously, NO metabolism during dialysis is a complex phenomenon, which is influenced by various factors. Our results are in agreement with, but also elaborate on the data presented in the short

communication of Jamil, who also found a low NO synthesis during cool dialysis.[27]

The fact that NO synthetic capacity and the reduction in blood pressure were both less pronounced during cool dialysis compared to dialysis-37.5°C does not necessarily imply a causal relationship. Still, the fact that various authors have reported that NO is related to hemodialysis hypotension[11-14,17] suggests that our findings might contribute to the understanding at a more basic level for the apparent relation between hemodynamic stability and extra corporeal energy balance observed in earlier studies.[4,6-9] Changes in NO synthetic capacity did not correlate with changes in blood pressure in the present study. Nevertheless, this is not unexpected because the decline in blood pressure during dialysis is not only due to changes in arterial or venous tone, but occurs as an interplay between a decline in blood volume, impaired vascular resistance and cardiac factors.[1] Moreover, in the present study, only stable patients were included. Thus, as observed previously by other authors, an increased NO synthesis may play an important role in hemodynamic instability in hypotensive prone dialysis patients.[11-14]
It is tempting to extrapolate some of the hemodynamic findings observed during heat stress in non-uremic humans to the changes in vascular reactivity observed during hemodialysis treatment without extra corporeal blood cooling. Both during heat stress and during hemodialysis, significant cutaneous vasodilatation occurs. Moreover. although this matter is still controversial[28,29], elevated NO levels were observed in patients with heat stroke[30]. and are believed to play an important role in the cutaneous vasodilatation during heat stress.[5] Of course, changes in core temperature during heat stroke are incomparable with those observed during dialysis treatment. However, NO dependent cutaneous vasodilatation under experimental heat stress was already observed after an increase in core temperature of 0.35°C, thus at values commonly observed during dialysis treatments.[31]

Various limitations of the present study should be discussed. Firstly, NO synthetic capacity was studied ex vivo by [^{3}H]-L citrulline formation from [^{3}H]-L arginine by human umbilical vein endothelial cells (HUVEC), after incubation with plasma samples obtained during the respective treatment sessions. Thus, NO synthetic capacity observed in the in-vitro situation may not be directly extrapolated to NO synthesis per se in the in-vivo situation, as the results may also be influenced by changes in NO modulators (inhibitors or stimulators) in the plasma samples. Still, although we did not measure levels of NO inhibitors such as asymmetric dimethyl arginine (ADMA) in the present study, it appears unlikely that the removal of substances such as l-ADMA would vary to a large extent between two dialysis sessions which only differ in dialysate temperature.

Moreover, absolute values for [^{3}H]-L citrulline formation from [^{3}H]-L arginine a the start of the treatment were not entirely equal at the start of both dialysis sessions. However, in view of the values obtained during the subsequen points in time, the inter-dialytic changes appeared to be consistent in both dialysate sessions. Moreover, relative changes showed the same trends.

Concluding, the stimulatory effect of uremic plasma on endothelial NO synthesis increased during dialysis-37.5°C, in contrast to cool dialysis. Whether this may contribute to differences in hemodynamic stability between standard and cool temperature dialysis deserves further study.

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Chapter 5

Pre-dilution on-line hemofiltration versus low-flux hemodialysis: a randomized prospective study

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Nephrology Dialysis Transplantation 2005;20:1155-63

Abstract

Background
Accumulation of larger molecular weight uremic toxins molecules may have a negative effect on the cardiovascular and nutritional state of dialysis patients, and influence uremic symptomatology. Their clearance can be enhanced by the use of hemofiltration (HF). Aim of the study was to compare the effects of low-flux hemodialysis (HD) (ultrapure dialysate; polyamide membranes) and pre-dilution on-line HF (1:1 blood/substitution ratio; target filtration volume 1.2 * body weight), on cardiovascular and nutritional parameters, inter-dialytic levels of uremic toxins, and quality of life (QoL; Laupacis questionnaire) during one year follow-up.

Methods
Forty patients were randomized for HF and HD and subsequently followed for one year. At regular intervals (t=0, after 6 months, after 12 months) cardiovascular (echocardiography, blood pressure measurements, puls wave velocity), nutritional parameters (Dual-energy X-ray absorptiometry (DEXA), insulin like grow factor-1 levels) and quality of life (questionnaire) was measured. Uraemic toxins as ß2M, l-ADMA complement factor D, leptin, advanced glycation end products and homocystein were measured during the study.

Results
Left ventricular mass index did not change in the HF (127 ± 33→131 ± 36g/m^2 after 12 months) and in the HD group (135 ± 34→138 ± 32 g/m^2), nor did pulse wave velocity or 48-hours systolic and diastolic blood pressure. Lean body mass, assessed by DEXA, increased in the HF (44.8 ± 8.9→46.2 ± 9.6 kg; $p<0.05$), but not in the HD group (49.4 ± 9.2→50.6 ± 8.8 kg). Insulin like growth factor-1 levels remained stable in the HF, but decreased in the HD group. QoL for physical symptoms improved in the HF (4.2 ± 1.2→5.0 ± 1.1;$p<0.05$) but not in the HD group (4.0 ± 1.0→4.4 ± 1.4). ß2M, complement factor D, and homocystein decreased significantly in the HF, but not in the HD group, whereas l-ADMA, leptin and advanced glycation end products did not change.

Conclusion
HF appeared to have beneficial effects on nutritional state, whereas no differences in cardiovascular parameters were observed compared to HD. Treatment with HF resulted in marked changes in the uremic toxicity profile and an improvement in QoL.

Introduction

Conventional hemodialysis (HD) is highly efficient in removing small molecular weight (MW) uremic toxins. However, the clearance of larger MW substances is limited. A greater removal of larger MW substances is achieved by convective therapies, such as hemofiltration (HF)[1]. Despite a burst of initial enthusiasm for HF during the late seventies and early eighties, interest in this technique gradually diminished. One of the reasons is the limited removal of small MW uremic toxins due to the relatively small filtration volumes used during conventional HF and the high costs of the prefabricated substitution fluids. However, so called on-line production of substitution fluid enables the production of bicarbonate-containing, sterile substitution fluid in large quantities, thereby circumventing limitations in small MW removal[1].
From a theoretical point of view, and based on results of retrospective studies with conventional HF, an increased removal of larger MW substances might have beneficial effects on blood pressure control and cardiovascular morbidity[2,3]. Moreover, a recent study with pre-dilution on-line HF showed an improvement in the quality of life[4].
With regard to cardiovascular disease, the prevalence of hypertension and left ventricular hypertrophy is notably high in hemodialysis (HD) patients. Recent data suggest that accumulation of the endogenous nitric oxide inhibitor L-asymmetric dimethylarginine (l-ADMA) might play a role in this respect[5]. Removal of l-ADMA (202 D) during conventional HD is limited[6]. Improved clearance of l-ADMA in combination with improved blood pressure control was observed during on-line hemodiafiltration (HDF)[7]. Homocystein, which is marginally removed by conventional hemodialysis[8], has also been implicated in the increased cardiovascular morbidity in dialysis patients. Lastly, it has been suggested that accumulation of advanced glycation end products (AGEs) might play a role in cardiovascular damage in HD. AGEs are inefficiently removed during HD, but a reduction in predialytic levels of AGEs was observed during HDF[9].
Another important factor in survival of patients on hemodialysis is their nutritional state. It has been hypothesized that accumulation of uremic toxins in the range of 2-5 kD or the toxin leptin may reduce appetite[10,11].

Prospective randomized studies on the potential benefits of convective therapies, using one-line preparation of substitution fluids, are however scarce[4,12-14] and did not yet focus on cardiovascular and nutritional parameters in detail. Moreover, most studies assessed HDF, during which the clearance of both smaller and larger molecular weight uremic toxins is enhanced[1]. As during pre-dilution HF, the highest convective clearance as yet possible can be achieved with a small molecular clearance nearly comparable to that of HD.

Making HF an interesting method to study the pathophysiological effects of an increased clearance of larger molecular weight uremic toxins per se.
The aim of the present prospective randomized study was to compare the effects of on-line predilution HF and low-flux HD on cardiovascular and nutritional parameters, quality of life and uremic toxicity profile.

Patients and methods

Patients

Approval for the study was obtained from the ethical committees of two centers; University Hospital Maastricht and VieCuri Medical Center; Venlo, both located in the Netherlands.
Included were patients, treated with low-flux HD three times weekly, who were on dialysis treatment for at least 3 months and had an adequate arteriovenous access. Excluded were patients with severe cardiovascular morbidity, defined as a left ventricular ejection fraction <25% and/or coronary heart disease NYHA classification of III-IV, and severe intercurrent illness. Patients were enrolled during a period of two years.
All patients gave written consent. Characteristics of the patients who entered the final randomization are displayed in table 5.1. In the HF group, original renal disease was nephrosclerosis/renal vascular disease in 4 patients, glomerulonephritis in 7 patients, diabetic nephropathy in 4 patients, polycystic disease in 1 patient, other diagnoses in 4 patients. In the HD group, original renal disease was nephrosclerosis/renal vascular disease in 3 patients, glomerulonephritis in 6 patients, diabetic nephropathy in 5 patients, polycystic disease in 3 patients, other diagnoses in 3 patients.

Table 5.1 Patient characteristics.

	Hemofiltration	Hemodialysis
n	20	20
Age (years)	59 ± 13	58 ± 12
Sex (m/f)	11 / 9	16 / 4
Body surface area (m^2)	1.74 ± 0.15	1.88 ± 0.19
Time on dialysis (months)	28 ± 16	24 ± 23
Diabetes mellitus	4	6
C-reactive protein (mg/l)	7.0 ± 7.0	12.2 ± 13.4
Serum calcium (mmol/l)	2.4 ± 0.2	2.4 ± 0.2
Serum phosphate (mmol/l)	1.8 ± 0.5	1.8 ± 0.4
Hemoglobin (mmol/l)	6.9 ± 0.7	7.1 ± 0.9
Parathormone (pmol/l)	14.8 ± 18.6	25.4 ± 39.1

Study design and treatment characteristics

The study was performed in two dialysis centers. Patients were randomized for pre-dilution HF or standard HD, and were stratified for diabetes. Patients who dropped out of the study within three months were replaced.
On-line HF was performed with the AK 200/200-S ULTRA (Gambro®, Lund, Sweden). HD was performed with AK 200 ULTRA or 4008H modules (Fresenius Medical Care®, Bad Homburg, Germany).
For the HF treatment polyamide (Polyflux 24S [Gambro®]) dialyzers were used. The HD group was treated with low-flux polyamide membranes (Polyflux 8L (Gambro®) and ultrapure dialysis fluid, manufactured through a double reverse osmosis unit and electrical deionisation, followed by filtration (U 8000 [Gambro®] or Diasafe [Fresenius Medical Care®]). The minimal target double-pool Kt/V during low flux dialysis was 1.2.
Treatment time was not changed during the study. The dilution of blood in HF was aimed at the ratio 1:1 and the total filtrate volume in the HF group was aimed at 1.2 times body weight[1]. The composition of both dialysate and substitution fluid was: sodium 140 mmol/l, potassium 2 mmol/l, calcium 1.50 mmol/l, bicarbonate 32-36 mmol/l, glucose 1 mmol/l, magnesium 0.5 mmol/l.

Power analysis

Left ventricular mass index (LVMi) was the primary outcome variable.
Assuming a standard deviation of 25 g/m² for changes in LVMi (extrapolated from)[15], a total number of 22 patients would needed to detect a difference of 25 g/m² between the effect of the different treatment modalities[16,17] with a type I error probability of 0.05 and a type II error probability of 0.10. In order to correct for multiple comparisons and expected drop-outs, we chose to include 40 patients because of expected drop-outs due to transplantation or death during the long follow-up time. Patients with withdrew from the study within 3 months (3 patients, due to transplantation) were replaced.

Treatment efficacy

Treatment efficacy was assessed by the urea reduction ratio and by measurement of Kt/V by partial dialysis quantification (DQM 100 Urea monitor; Gambro®; Lund Sweden).

Cardiovascular parameters

Blood pressure was measured using an ambulatory blood pressure device (Spacelabs Medical B.V., Utrecht, the Netherlands) during two days between

dialysis treatments, short interval. The measurement was performed before randomization, after six months and after one year.
Also, the prescription of antihypertensive medication was registered. For each patient a score was made, in which every medication used was given a score of 100. Adding another antihypertensive drug resulted in an increase of 100, cessation of a drug resulted in a decrease of 100. Changing the dose of an already used drug resulted in an increase of 50 after a raise in dosage or a decrease of 50 after a lowering of the drug. After assessment and potential correction of dry weight, antihypertensive agents would be reduced if pre-dialytic systolic blood pressure decreased below 110 mmHg and increased if pre-dialytic systolic blood pressure consistently increased above 160 mmHg.
Two-dimensional echocardiography was performed on a midweek inter-dialytic day using a HP Sonos 5500 ultrasound system (Hewlett Packard®, Palo Alto, USA). Left ventricular mass was calculated according to the formula of Devereux and Reichek[18].
Arterial stiffness was assessed by Pulse Wave Velocity (Complior SP®, PMS instruments, Berkshire, United Kingdom), also on a midweek inter-dialytic day. All these measurements were repeated after 6 months and after one year.

Nutritional state.

The energy and protein intake was assessed using a 7 days questionnaire. Protein catabolic rate was assessed by the DQM Urea monitor (Gambro®, Lund, Sweden). Subjective global assessment on a seven-point scale was performed.
Dual-energy X-ray absorptiometry (DEXA) was performed for the measurement of body composition by a QDR 4500 densitometer (Hologic Inc. Waltham, MA, USA). These investigations were performed on a midweek inter-dialytic day before randomization and after six months and one year.

Fluid status

Dry weight was adjusted on clinical criteria, aided by echography of the inferior caval vein. Fluid status was assessed at baseline and after 6 and 12 months by multifrequency bioimpedance analysis (Xitron 4000®; San Diego; USA). Detailed methodology for the latter technique is described elsewhere[19].

Quality of life.

The kidney disease questionnaire of Laupacis[20] was used to evaluate the quality of life (QoL). Patients were asked to identify their specific physical problems, next to questions regarding frustration, depression and well-being. Patients were asked to name their complaints on a scale ranging from

1 (severe) to 7 (none). The questionnaire was performed before randomization and was repeated after six months and after one year.

Laboratory analysis

The following routine parameters were recorded before randomisation, after six months, and after one year: hemoglobin level, C-reactive protein, albumin, serum calcium, phosphorus and parathyroid hormone. At the same time intervals, noradrenalin, endothelin and insulin-like growth factor-1 (IGF-1) and markers for oxidative stress were assessed. All blood samples were taken on a midweek inter-dialytic day. The following uremic toxins were assessed before randomization and after 6 months: ADMA, homocystein, leptin, complement factor D and β2M, and AGE-related fluorescence (AGEs).
Complement factor D was measured by an enzyme-linked immunoassay using two monoclonal antibodies directed against human complement factor D[21]. ß2-M was assessed by a commercial enzyme-linked immunoassay (Immun-diagnostik, Bensheim, Germany, detection limit 0.1 mg/l) using a polyclonal antibody directed against human ß2-M. Total (free and protein-bound) homocystein was analyzed by isocratic reversed phase HPLC with fluorescence detection. ADMA was analyzed by capillary electrophoresis with fluorescence detection. IGF-1 was determined with an immunoradiometric assay (IRMA) with kits from Nichols (Bad Vilbel, Germany). Leptin measurements were performed with a radioimmunoassay (Mediphos Medical Supplies (Renkum, The Netherlands). Noradrenalin was measured with cation-exchange high performance liquid chromatography using electrochemical detection. For the study of antioxidants (Total Antioxidant State, glutathion peroxidase and superoxide dismutase) kits from Randox (Crumlin, UK) were used and measured on a Cobas Mira (Radiometer, Copenhagen, Danmark).
The determination of AGEs was performed by quantitative total fluorescence analysis[22].

Statistical analysis

Changes in measured variables with time were assessed by repeated measures ANOVA, with the mode of treatment (HF or HD) as a between-subjects factor. If changes within groups were significant, differences were further analyzed using the paired student-t test. Data were analysed using SPSS version 12.01. p-values below 0.05 were considered significant.

Results

After randomization, 20 patients enrolled the HF group and 20 patients enrolled the HD group. Thirty-six patients completed the six-months period (HF:19 patients; HD: 17 patients). After one year 27 patients were eligible for analysis (HF: 13 patients; HD: 14 patients).
During the study period, two patients died, one after a rupture of an aortic aneurysm (HF group) and one due to an acute myocardial infarction (HD group). Four patients were transplanted (HF group 1 patient; HD group 3 patients). During the study in total 3 patients (all HF) were withdrawn for medical reasons: one patient was starting treatment with chemotherapy for newly diagnosed cerebral and ossal metastasis of mamma carcinoma, one patient after a traumatic (not dialysis related) myelum compression and one patient after abdominal sepsis. One patient (HF) had an allergic reaction to the dialyzer. Three patients (1 HF; 2 HD) decided to withdraw from the study for personal reasons.
Achieved Kt/V, measured by partial dialysis quantification, and urea reduction rate were not significantly different between the HD and HF groups. Urea reduction rate in the HF group was 0.70 ± 0.08 at baseline (thus before the start of the study), 0.67 ± 0.07 at 6 months and 0.66 ± 0.05 at 12 months. In the HD group, urea reduction rate was 0.68 ± 0.05% at baseline, 0.67 ± 0.07% at 6 months and 0.67 ± 0.07% at 12 months. In the HF group, Kt/V was 1.38 ± 0.32 at baseline, 1.33 ± 0.21 at 6 months and 1.14 ± 0.13 at 12 months. In the HD group, Kt/V was 1.41 ± 0.04 at baseline, 1.34 ± 0.20 at 6 months and 1.36±0.26 at 12 months.

Cardiovascular parameters

No significant changes in echographic parameters (LVM, figure 5.1), pulse wave velocity and blood pressure (figure 5.2) were observed in the study, neither in the HF, nor in the HD group (figure 5.1 and 5.2). The change in LVMi in the HF group during the one year period was 4 ± 20 g/m^2.
Data are summarized in table 5.2
Number of antihypertensive agents and medication score also did not change significantly, although there was a tendency towards a rise in both groups. In eight patients (5 HF and 3 HD patients), angiotensin converting enzyme inhibitors were started during the study period and were withheld in two patients (1 HF and 1 HD patient). Noradrenalin levels decreased significantly both in the HD and HF groups, but were not significantly different between both groups. Extracellular water remained constant during the study period (table 5.3).

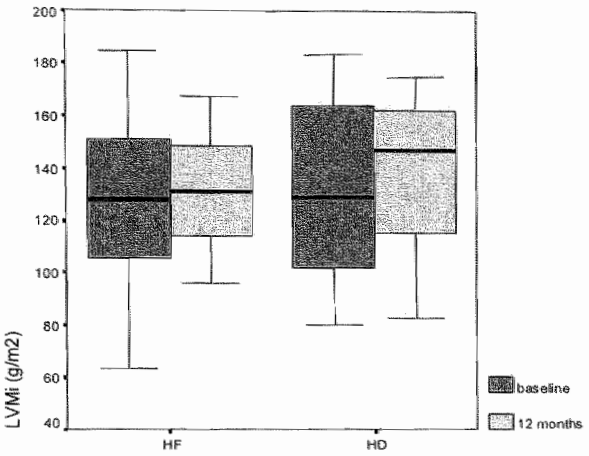

Figure 5.1 Change in left ventricular mass index in the HF and HD groups,
HF: hemofiltration; HD: hemodialysis; LVMi=left ventricular mass index.
Box plots indicate 25th and 75th percentiles (thick line is median value), capped bars indicate minimum and maximum values, circles indicate outliers.

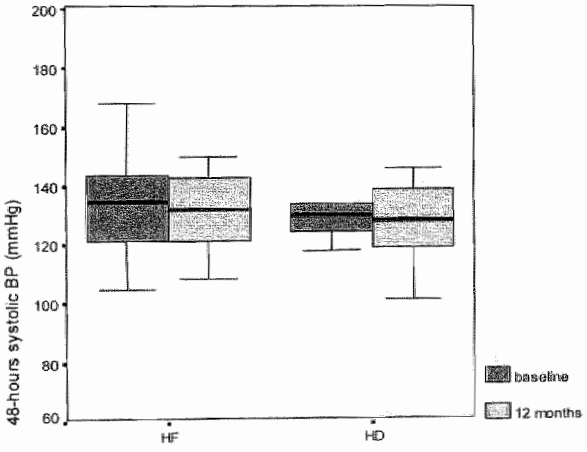

Figure 5.2 Change in 48-hours ambulatory blood pressure in the HF and HD groups.
HF: hemofiltration; HD: hemodialysis; LVMi=left ventricular mass index.
Box plots indicate 25th and 75th percentiles (thick line is median value), capped bars indicate minimum and maximum values, circles indicate outliers.

Nutritional parameters

Data are summarized in table 5.3. Body weight did not change significantly during the study. In contrast, lean body mass increased significantly in the HF group, but not in the HD group, although differences between groups did not reach significance. IGF-1 decreased significantly in the HD group during the study period. In the HF group, IGF-1 levels decreased initially between the 0 and 6 months period, but increased significantly again during the 6 and 12 months period. The difference in IGF-1 levels between the HD and HF groups was significant. No differences in serum albumin, protein catabolic rate, nor in protein and energy intake assessed by dietary questionnaire were observed between the groups (data not shown). Of note, only 10 patients in the HF group and 6 patients in the HD group completed all 7 days questionnaires at all points in time.

Quality of life

QoL, with regard to physical symptoms, improved significantly in the HF group (4.1 ± 1.4 at baseline, 5.4 ± 0.9 after 6 months and 5.0 ± 1.1 after 12 months; $p<0.01$), but not in the HD group (4.2 ± 0.9 at baseline, 4.3 ± 1.2 after 6 months and 4.4 ± 1.4 after 12 months), with differences between both groups approaching significance ($p=0.06$). QoL for other aspects did not change significantly in any treatment group.

Uremic toxicity profile

Data are summarized in table 5.4. β2M decreased significantly in the HF, but not in the HD group with significant differences between both groups, the same holding true for complement factor D. Homocystein decreased significantly in the HF, but not in the HD group, whereas the difference was not significant between both groups. ADMA, leptin, and AGE related fluorescence did not change in any group. Of note, leptin levels were significantly related to fat mass ($r=0.65$; $p<0.01$), assessed by DEXA, but also inversely to protein and energy intake ($r=-0.44$ and $r=-0.48$; $p<0.05$).

Other parameters

Hemoglobin levels, serum albumin, calcium and phosphate, parathormone did not change within or between groups (data not shown). The same held true for C-reactive protein levels (7.0 ± 7.3 mg/l at baseline and 3.7 ± 3.7 mg/l after 12 months in the HF group and 11.8 ± 10.5 mg/l at baseline and 15.3 ± 17.1 mg/l after 12 months in the HD group). Total antioxidant status decreased

significantly in the HF group (1.34±0.10 mmol/l at baseline; 1.29±0.1 at 6 months and 1.21±0.20 at 12 months; $p<0.05$), but not in the HD group (1.38±0.16 mmol/l at baseline; 1.29±0.1 at 6 months and 1.30±0.14 at 12 months; $p<0.05$), without significant differences between both groups. However, superoxide dismutase did not change significantly, neither in the HF group (806±259 U/gram hemoglobin at baseline; 850±215 at 6 months and 978±240 at 12 months), nor in the HD group (799±167 U/gram hemoglobin at baseline; 860±126 at 6 months and 881±166 at 12 months; $p<0.05$).
The same held true for glutathion peroxidase, which did not change significantly, neither in the HF group (1131±339 U/mmol hemoglobin at baseline; 1050±399 at 6 months and 1071±344 at 12 months), nor in the HD group (1024±236 U/mmol hemoglobin at baseline; 973±307 at 6 months and 1088±322 at 12 months; $p<0.05$).

Table 5.4 Uremic toxins.

	Hemofiltration		Hemodialysis		P time	mode
	0	6 months	0	6 months		
ß2M (mg/l)	43.1± 18.0	20.4± 10.1*	39.2± 18.7	42.8± 17.1	<0.01	<0.01
complement factor D (mg/l)	28.0± 6.5	22.6± 8.5*	27.7± 7.9	29.8± 8.6*	<0.05	<0.05
homocystein	25.4± 7.4	21.1± 7.0*	29.7± 8.2	27.0± 9.3	<0.05	ns
leptin (µg/l)	24.4± 23.4	25.7± 28.7	24.9± 30.0	17.3± 24.8	ns	ns
ADMA (µmol/l)	1.37± 0.42	1.22± 0.38	1.61± 0.09	1.60± 0.55	ns	ns
AGE-fluorescence (mV/µl)	13.4± 3.2	12.2± 3.9	13.4± 4.9	10.6± 3.2	ns	ns

ß2M= beta 2 microglobulin; ADMA=L-asymmetric dimethylarginine; AGE=advanced glycation end products.
*$p<0.05$ compared to baseline.

Discussion

In the present randomized study, the effect of pre-dilution HF, using on-line preparation of substitution fluids on cardiovascular and nutritional state, quality of life, and the uremic toxicity profile was compared with low-flux HD using ultrapure dialysate and biocompatible membranes.
With regard to cardiovascular parameters, no significant differences in changes in LVMi, arterial stiffness and 48-hours ambulatory blood pressure measurements were observed between HD and HF. No study as yet assessed changes in cardiac parameters during HF. Comparing our data with the only published study which assessed cardiac effects of convective techniques, our data are in contrast to those of Schrander, who observed a significant decline in LVMi during a follow up period of one year in patients treated with acetate

free biofiltration (AFB), and an increase in LVMi in patients treated with HD[17]. The reason for the discrepant results between their study and the present one is not clear. Convective clearance is much larger during HF as compared to AFB, which is a modified hemodiafiltration technique. It should be noted that volume status, which can have a major influence on cardiovascular parameters, was meticulously controlled and checked in the present study. In our HD patients, LVMi remained stable during the follow-up period.

The absence of changes in structural cardiovascular parameters should be interpreted with caution, as the follow-up time of our study was relatively short, only one year, and the number of included patients relatively small. However, pronounced changes were observed in even smaller groups of dialysis patients and/or during shorter follow-up periods during quotidian HD or when using angiotensin receptor antagonists[16,17,23,24].

With regard to blood pressure control, our data are in contrast to those obtained by Altieri[4,15]. Whereas in our study, inter-dialytic blood pressure control did not differ between the HF and HD groups, Altieri observed an increase in ambulatory blood pressure during pre-dilution on-line HF compared to high-flux HD[4,15]. However, in this study, reactance and resistance assessed by bioimpedance decreased during the HF period, suggesting an increase in body hydration, whereas the prevalence of patients on antihypertensive treatment appeared to be lower in the HF treatment period[4]. However, the same authors also observed an increase in inter-dialytic blood pressure during HF compared to HDF, without changes in bioimpedance parameters or antihypertensive agents[15]. The increase in inter-dialytic blood pressure during pre-dilution on-line HF was interpreted by Altieri as a beneficial stabilizing effect of the increased convective clearance, contributing to a reduction in intra-dialytic hypotension[4,15]. However, also an increased sodium sieving has been observed during HF, due to coating of negative loaded proteins to the hemofilter[25]. Nevertheless, we and others were not able to confirm either a reduced sodium removal or increased sodium sieving during HF[26,27].

Thus, the discrepant results from the literature remain as yet unexplained. Data on the effect of HDF on blood pressure control are also conflicting[7,12,13,15,17]. Of note, only in few studies[4,15], including the present one, objective information on fluid state is available, and only some used ambulatory blood pressure monitoring[4,7,15]. All these discrepant results underline the necessity of strictly standardized procedures for blood pressure analysis and meticulous control of fluid state.

No significant differences in l-ADMA levels were observed between the HF and the HD group. Noradrenaline levels decreased in both the HF and HD groups. The mechanism behind this observation is not clear, but might be related to the fact that the prescription of angiotensin converting enzyme inhibitors tended to increase during the study period.

Regarding nutritional state, a small but significant increase in lean body mass was observed *within* the HF group, without significant changes in extracellular water. However, no significant differences were observed *between* the HF and HD groups, and these findings should thus interpreted with great caution. Still, significant differences between both groups were observed with regard to IGF-1 levels. The mechanism for the potential benefits of HF treatment on nutritional state remains to be elucidated. Changes in leptin levels were not observed, which may be due to the fact that also in renal patients, leptin levels are predominantly dependent upon fat mass[28], as also shown in the present study. Interestingly however, serum leptin levels were weakly, but significantly inversely related to energy and protein intake in our patient cohort.

During HF, no major changes in energy or protein intake were observed. These data should be interpreted with great caution, as various patients did not complete the 7 days questionnaires. However, also protein catabolic rate did not change. Our data are to a certain extent in contrast with those of Wizemann, who did not observe differences in nutritional status between patients randomized to HDF or low-flux HD, although in this study, body composition was not assessed in detail[13].

In agreement with Altieri[4], we observed an increase in the QoL of patients in the HF group, due to a general improvement in physical well being. However, it should be noted that patients were not blinded to the treatment modality.

C-reactive protein levels, did not change in the HF and the HD groups. It has also been suggested that convective therapies, as HDF and HF, might result in a reduction of the inflammatory response in dialysis patients.[29] However, comparisons between HD and HF have been complicated by the fact that in earlier studies hemodialysis was often performed with cuprohane membranes and contaminated dialysate were used, which may itself elicit an inflammatory response, whereas more biocompatible membranes and sterile substitution fluid was applied during hemofiltration. In the present study, HD was performed we so-called ultrapure dialysate with minimal bacteriological contamination. However, it should be noted that C-reactve protein was not assessed by a highly sensitive method and the variation of C-reactive protein levels was large. Nevertheless, intercurrent comorbid events may be more important for the variation of C-reactive protein levels than the dialysis modality[30,31].

Regarding the uremic toxicity profile, major improvements were observed in the HF group. β2M levels, obtained at an inter-dialytic day, decreased by almost 50%, which is far larger compared to earlier studies using HDF and is likely due to the higher convective clearance[12,32,33]. In addition, a significant decrease in complement factor D, which stimulates the alternative route of complement but inhibits the degranulation of polymorphonuclear leukocytes was observed[34]. In our study, AGEs did not change during HF, in contrast to the findings of Lin, who observed a decline in total AGE levels in patients treated with HDF

compared to low-flux dialysis[9]. Differences in methodology to assess AGE products may contribute to discrepant results between different studies[35].
A significant decline in homocystein levels was observed in HF treated patients, which is in accordance with data obtained in patients treated with so-called superflux dialysis[8].

Lastly, total antioxidant status declined slightly, but significantly within the HF group, although the difference with the HD group was not significant. The meaning of this observation is unclear, as the antioxidants superoxide dismutase and glutathion peroxidase did not decrease during HF. However, it is of interest that Morena observed an increase in vitamin C removal in patients treated with HDF[36]. Whether this is the explanation of the finding observed in our study remains to be elucidated.

Drawbacks of the present study are, already mentioned, the relatively small follow-up period and number of included patients. However, as mentioned previously, major changes in cardiac parameters have been observed with smaller number of patients or shorter follow-up periods with other interventions. Moreover, the standard deviation for the primary outcome variable (i.e., change in LVMi) was comparable to the assumption made in the power analysis. However, certainly in view of the beneficial effects of HF treatment on the uremic toxicity profile, larger studies are definitely needed to identify the potential role of convective therapies in the treatment or prevention of cardiovascular morbidity in the dialysis population.

Concluding, in this prospective randomized study, no significant differences in cardiovascular parameters were observed between patients treated with pre-dilution on-line HF compared to patients treated with low-flux ultrapure HD. However, small but significant improvements were observed in the nutritional state of HF group. In addition, treatment with HF resulted in a significant improvement in the physical well being of dialysis patients and in major improvements in the uremic toxicity profile.

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Chapter 6

Determinants of arterial distensibility in patients with renal failure

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Nephron Physiology 2003;95:43-48

Abstract

Background
An increased stiffness of the arterial system is an adverse risk factor for outcome in patients with renal disease. Few studies have focused on the determinants of an increased arterial stiffness in patients with renal failure. As the percentage of patients with renal failure secondary to vascular disease and /or diabetes mellitus is rapidly growing, and the underlying disease per se may also influence arterial wall properties, it may also be of interest to study arterial wall properties in relation to the etiology of kidney disease.

Methods
Distensibility coefficient (DC) of the common carotid artery [Walltrack®) was used as a marker of arterial stiffness. 117 patients were studied: 47 patients (age 63±10 years) with renal failure secondary to vascular disease and/or DM and 70 patients (age 57±13 years) with other diagnoses. The origin of renal failure was retrieved from the patients' charts.

Results
Age, mean arterial pressure and the serum calcium level were each independent predictors of DC. DC was significantly lower in the patients with vascular renal disease or DM [11.0±5.5 (1/MPa)] compared to patients with renal/urological disease [15.4±7.5 (1/MPa)]. Nevertheless, after correction for potentially confounding variables, the relation between cause of renal disease and DC lost significance in the overall group, but remained significant ($p<0.05$) in the younger age groups (≤61 years; median age of patient group).

Conclusion
Age, mean arterial pressure and the serum calcium level were the most important predictors of DC in our patients with renal failure. Only in younger dialysis patients, the origin of renal failure was an independent predictor of arterial wall stiffness.

Introduction

An increased stiffening of the large arteries, characterized by a reduced distensibility or compliance, or an increased pulse wave velocity, are common in hemodialysis (HD) patients, but also in patients with less advanced renal disease[1,2]. The relation between arterial wall abnormalities and cardiovascular morbidity and mortality[3,4] highlights the importance of this phenomenon. This relation can partly be explained by the fact that a reduction in the buffering capacity (compliance) of the arterial system leads to an increased systolic pressure load, and therefore to left ventricular hypertrophy. The latter is also an important predictor of mortality in patients with renal failure[5].
The cause of vascular abnormalities in renal patients is probably multifactorial. Potentially important contributing factors include hypertension, abnormalities in the calcium phosphate product and the level of renal function per se[1,2]. Another factor that is not widely considered in the evaluation of arterial wall abnormalities in renal patients is the underlying cause of renal insufficiency. The number of patients with renal failure due to vascular renal disease or diabetes mellitus (DM) is rapidly increasing[7]. From a theoretical point of view, the underlying disease might have a strong influence on arterial wall properties, apart from the effects of renal disease itself[8]. Nevertheless, few data are present in literature on the potential influence of the cause of kidney disease on arterial stiffening. It is hypothesized that arterial distensibility coefficient (DC) will be lower in patients renal failure secondary to vascular disease and/or DM compared to patients with other diagnoses.
The goal of the present study was therefore to assess determinants of DC of the common carotid artery, as a marker of arterial stiffness, and to compare DC between patients with renal failure secondary to vascular disease and/or DM and patients with other diagnoses, taking potentially confounding variables into account.

Methods

Study design

In this cross-sectional study, determinants of the DC of the common carotid artery were assessed in a cohort of patients with renal failure. Also, DC was compared between patients with renal failure due to vascular disease and/or DM compared to patients with other diagnoses. As vascular diseases were considered nephrosclerosis, diabetes mellitus, cholesterol emboli, and renal artery stenosis. Diagnoses were retrospectively retrieved from the patients'

record. The protocol was approved by the local ethics committee and all patients gave written informed consent.

Patients

Inclusion criteria were the presence of renal failure, arbitrarily defined as a creatinine clearance below 60 ml/min. Exclusion criteria for the study were: cardiac failure (NYHA III and higher), coronary heart disease (NYHA III and higher), symptomatic stenosis of the carotid artery, uncontrolled hypertension (systolic blood pressure higher than 180 mmHg or diastolic blood pressure higher than 105 mmHg), and acute renal insufficiency. Eligible patients from two regional hospitals were asked to participate in the study (Kuratorium für Heimdialyse; Würselen; Germany; St. Elisabeth Hospital Tilburg, the Netherlands).
In patients with renal failure according to vascular disease and/or DM (n=47), the origin of renal failure was nephrosclerosis/renal artery stenosis (69.5%), DM (23.9%), and cholesterol emboli (6.5%). Of the patients 45.7% were treated with hemodialysis, 28.3% with peritoneal dialysis (PD), whereas 26.1% of the patients had chronic renal failure not yet treated with renal replacement therapy. Mean creatinine clearance in the CRF patients was 27.8±9.3 ml/min. Other patient characteristics are displayed in table 6.1.
In patients with other diagnoses (n=70), the origin of renal failure was chronic glomerulonephritis (48.6%), polycystic disease (15.3%), multiple myeloma (4.2%), chronic pyelonephritis (12.5%), scleroderma (2.8%), unresolved acute tubulus necrosis (2.8%), nephrolithiasis (4.2%), and interstitial nephritis (5.6%), whereas hemolytic uremic syndrome and postobstructive renal disease, each occurred in 1 patient. 45.8% of the patients were treated with hemodialysis, 29.2% with peritoneal dialysis, whereas 25.0% of the patients had chronic renal failure (CRF) not yet treated with renal replacement therapy. Mean creatinine clearance (Cockroft formula) in the CRF patients was 37.9 ± 18.9 ml/min (not significantly different from patients with renal vascular disease and/or DM

Measurement protocol

DC of the right common carotic artery (CCA) was measured after a period of 15 minutes supine rest at room temperature. Measurements were performed in the recumbent position. Patients were asked not to smoke, eat or drink starting 4 hours before the measurements were performed, although for obvious reasons patients were allowed to take their regular medication.
The distension and diameter of the right CCA was assessed using an automated echo-tracking system (Wall Track® II, Pie Medical, the Netherlands)[11,12]. With this system the vessel walls are detected with the use of ultrasound, using a 7.5 MHz linear array echo-probe. A sample volume is

placed at the posterior and anterior walls of the CCA, 1 to 2 cm proximal of the bulbus. The raw radiofrequency data are subsequently stored in a computer, after which the vessel wall motion during each heart cycle is tracked with the electrocardiographic trigger as reference time-point. In this way the distension (ΔD) from diastole to systole, and the diameter (D) of the artery at end-diastole can be calculated. ΔD was expressed as the mean of at least 7 consecutive reproducible cardiac cycles. DC was computed according to the following formula[11]: $DC = 2(\Delta D/D)/\Delta P$, where the pulse pressure (ΔP) was obtained in the brachial artery.

Dialysis schedule

HD patients, scheduled on a maximum of one day between subsequent dialysis sessions, were measured directly before dialysis, because measurements after dialysis may be disturbed by the rapid changes in fluid and/or electrolyte status. Dialysis schedules were changed if necessary to perform measurements in the morning, such to avoid diurnal variations in the measured arterial wall properties[9]. All HD patients were dialyzed with a dialysate calcium concentration of 1.50 mmol/l. All HD patients and PD patients were treated according to the DOQI guidelines, with respect to dialysis efficacy and treatment of anemia[10].

Blood pressure

Arterial blood pressure (systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP)) were measured with an automatic blood pressure monitor at the brachial artery (Dinamap 1486 SX®, Critikon, Florida, USA) every 3 minutes. In healthy controls, CRF, and PD patients the right brachial artery, whereas in HD patients, the brachial artery contralateral to the arteriovenous fistula was used. Pulse pressure (ΔP) was calculated as SBP minus DBP.

Laboratory parameters

In the patient groups, blood samples were taken for assessment of hemoglobin (Coulter-Genis®, California, U.S.A.), calcium, phosphate, (all Syncron LX 20®, Beckman Coulter, California, U.S.A.), parathormone (PTH) (IRMA assay®, Nichols Institute Diagnostics, California, U.S.A.).

Statistical analysis

Differences between the groups were either analyzed by the Student t-test for independent samples or by the Pearson chi-square test. All metric values are

expressed as mean ± SD. Correction for confounding variables was performed using multiple regression analysis with DC as a dependent variable. In this analysis, the "cause of renal failure" was coded as a dummy variable. First, a basic regression model was searched for, in which all effects had p values <0.05. Next, first order interactions of pairs of predictors within the basic model were tested for significance by forward selection. If interactions turned out to be significant, a split regression model analysis was done to test the original DC difference for "cause of renal failure"-factor within subpopulations of patients. A p value less than 0.05 was considered to be statistically significant. All calculations were made using SPSS-pc programmes (version 11.0.1, SPSS® inc., Chicago, Ill.).

Results

DC appeared to be significantly lower in patients with renal failure secondary to vascular disease and.or DM compared to patients with other diagnoses: 11.0 ± 5.5 1/MPa in patients with renal failure secondary to vascular disease and/or DM and 15.4 ± 7.5 1/MPa in patients with other causes of renal failure (p=0.001) (table 6.1 and figure 6.1).
In multiple regression analysis, the following predictors were included as potential predictors for DC: age of the patient, gender, MAP, serum calcium, use of antihypertensives and the use of angiotensin converting enzyme blockers (or angiotensin receptor antagonists). Age and gender of the patient were always included in the basic model, whether they had a significant or nonsignificant relationship with DC.
Significant predictors for DC were age, MAP, and calcium. Variance explained for "origin of renal disease" added to this model is 0.006, F=1.65 (p=0.20), and total variance explained for all 5 predictors is 0.635. So, the original difference for the "origin of disease" groups is explained notably by MAP and age.

Table 6.1 Patient characteristics.

	other	vascular/DM	p
n	70	47	
Age (years)	56.5 ± 13.0	62.8 ± 10.0	0.006
DC (10^{-3}/kPa)	15.4 ± 7.5	11.0 ± 5.5	0.001
MAP (mmHg)	104.3 ± 17.7	107.9 ± 17.0	0.09
ACE/AT-II (nr/patient)	0.36 ± 0.48	0.30 ± 0.47	ns
Antihypertensives (nr/patient)	1.5 ± 1.1	1.7 ± 1.1	ns
Parathormone (pmol/l)	19.3 ± 3.0	14.0 ± 17.0	ns
Setum phosphate (mmol/l)	1.8 ± 0.5	1.5 ± 0.5	0.006
Hemoglobin (mmol/l)	7.3 ± 0.9	7.1 ± 1.1	ns
Serum calcium (mmol/l)	2.4 ± 2.5	2.5 ± 1.8	ns

ACE=angiotensine converting enzyme inhibitors; AT-II= angiotensine receptor blockers.

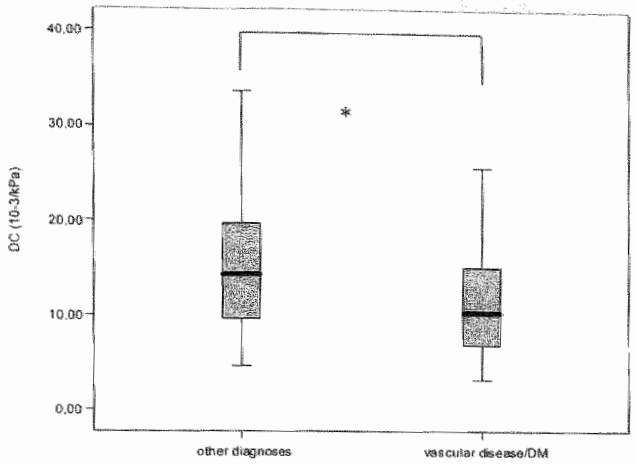

Figure 6.1 DC in relation to origin of renal disease. Values expressed as box-plots. Box indicates the 25th-75th percentile range (line in box=median). Capped bars indicate the 10th-90th percentile range. *p<0.05.

In each of the 10 possible first order interactions were added to this basic model, only the interaction between disease and age turned out to be statistically significant: variance explained by adding this to the basic model is 0.016, F=4.82 (p=0.03). Table 6.2 gives the regression coefficients, t-values, and p-values for this extended regression model. To get a better understanding of this interaction effect, the patient population was split half around its median value of age into 2 groups: 57 patients with age ≤61 years and 60 patients with age>61 years. Regression analysis was now twice repeated, first for patients ≤61 years of age (table 6.3). Here, adding the "disease" group to the (possible) predictors shows a significant addition to the variance explained of DC (F=4.28; p=0.04) (table 6.4). Hence, the original relation between DC and origin of disease is maintained, i.e younger patients with secondary renal disease show a lower DC than patients with primary renal disease. However, in the patients older than 60 years, this relationship turned out to be nonsignificant (F=0.37; p=0.5). (table 6.4).

Table 6.2 Results of the eventual multiple regression model with dependent variable DC. Variance explained: 0.651.

Model	Unstand. Coefficient B	Std. Error	Standard. Coefficient Beta	t	Sig.
	63.583	5.517		11.525	<0.001
Disease#	-11.506	4.790	-0.786	-2.402	0.018
Age#	-0.407	0.041	-0.701	-9.818	<0.001
Gender	-0.598	0.876	-0.040	-0.682	0.497
MAP	-0.107	0.018	-0.351	-5.903	<0.001
Ca	-0.0426	0.019	-0.131	-2.207	0.030
Dis*Age	0.171	0.078	0.743	2.196	0.030

Disease (0=Other,1=Vascular/Diabetes Mellitus), Gender (0=male,1=female), Ca=serum calcium. Dis*Age is first order interaction of Disease and Age.
t values not interpretable because of hierarchy effects after inclusion of first order interaction for disease*age.

Table 6.3 Results of the eventual multiple regression model with dependent variable DC for patients of 61 years of age and less. Variance explained: 0.420.

Model	Unstand. Coefficient B	Std. Error	Standard. Coefficient Beta	t	Sig.
	51.360	9.612		5.343	0.000
Gender	-2.129	1.709	-0.136	-1.246	0.219
MAP	-0.166	0.037	-0.505	-4.480	0.000
Ca	-0.0375	0.039	-0.107	-0.952	0.345
Disease	-3.840	1.856	-0.225	-2.069	0.044

Disease (0=Other,1=Vascular/Diabetes Mellitus), Gender (0=male,1=female), Ca=serum calcium.

Table 6.4 Results of the eventual multiple regression model with dependent variable DC for patients older than 61 years of age. Variance explained: 0.132.

Model	Unstand. Coefficient B	Std. Error	Standard. Coefficient Beta	t	Sig.
	26.392	6.740		3.916	0.000
Gender	0.718	1.184	0.083	0.606	0.547
MAP	-0.0412	0.023	-0.237	-1.784	0.081
Ca	-0.0421	0.024	-0.230	-1.720	0.092
Disease	-0.681	1.113	-0.085	-0.612	0.544

Discussion

Age, baseline MAP, and to a lesser degree, the serum calcium level , were the most important predictors of DC in the present cohort of patients with renal failure. Also, in the present study, DC of the common carotid artery was significantly lower in patients with renal failure secondary to vascular disease

and/or DM compared to patients with other renal diagnoses. The relation between origin of renal disease and DC lost significance after correction for confounding variables in the overall group. Only in younger patients, the origin of renal disease remained a significant independent predictor for DC.
It can be hypothesized that especially in the younger patients with renal failure secondary to vascular disease and/or DM, the presence of generalized vascular disease in the will have an independent effect on arterial stiffness, which is overruled in the older patients by the contribution of other factors.
Remarkable was the very strong effect of age itself on DC in this heterogeneous patient group, which is in accordance with earlier studies in patients in healthy subjects and in patients with renal disease[1,13,14]. The importance of this finding stresses the fact that groups should be strictly age-matched when arterial wall properties are to be compared.
Apart from age, blood pressure was also significantly related to DC of the common carotid artery. Blood pressure and arterial wall properties may interact in the sense that on one hand, an increase in blood pressure may result in structural and functional changes of the arterial wall. On the other hand, an increased stiffness in conduit arteries will result in an augmentation in systolic blood pressure due to a reduced buffering of the pulsatile outflow from the ventricle[15].
Serum calcium levels were inversely related to DC of the common carotid artery, which is in line with earlier studies describing a relation between vascular calcifications or serum calcium levels with arterial stiffness[16-18]. The present study is therefore in agreement with emerging insights regarding the detrimental effects of calcium on arterial wall properties in dialysis patients[19].

An obvious drawback of the study is the fact that the diagnosis of the patient was retrieved by examination of the charts. In many patients, a clinical diagnosis was made without availability of renal biopsy data, which is an inevitable consequence of a clinical observational study like the present one. Moreover, the retrospective character of the study precludes definite conclusions about the real relationship between DC and origin of renal disease. To overcome the problems of confounding variables, like age of the patients and MAP, we used multiple regression analysis to control for these disturbing effects in a statistical way.
It should be noted that patients with diabetes mellitus and renal vascular disease were analyzed concomitantly, which ideally should have been analyzed separately. Nevertheless, in view of the relatively small amount of diabetic patients in the present sample, this approach was not possible for reasons of power.
A technical limitation is the fact that pulse pressure, which is needed for the calculation of DC, was not measured at the site of the arterial wall.

Theoretically, a reliable assessment of distensibility requires the measurement of pressure at the site of determination and for this goal, applanation tonometry is advocated by some authors. Nevertheless, even applanation tonometry itself is not free of drawbacks[20]. Recently the use of the distension wave, which was found to be a good alternative for the applanation tonometry, has been advocated[12]. In another study, we used this technique to assess the local pulse pressure at the site of measurement at the CCA in a subset of patients and observed a highly significant correlation between this method and the method subsequently used in this study[1].

Concluding, age, blood pressure and the serum calcium level were the most important predictors of DC in our cohort of patients with renal failure. Only in younger patients, the origin of renal disease, was an independent predictor of arterial wall stiffness.

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Chapter 7

C-reactive protein levels in dialysis patients are highly variable and strongly related to co-morbidity

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Nephrology Dialysis Transplantation 2003;18:221

Abstract

Background
Although the relation between C-reactive protein (CRP) levels and outcome in dialysis patients is well established, data on the relation between changes in CRP levels and intermittent morbidity are scarce, as are data on the variability of CRP levels. Aim of the present study was to observe the variability of CRP levels and also to make an attempt to link elevated CRP levels to intermittent clinical events.

Methods
CRP levels and detailed clinical data were assessed during a monthly interval in all dialysis patients of our unit (n=60) during a three months period.

Results
The correlation coefficient between CRP levels at the start of the study and one month later was r=0.55 ($p<0.01$) and 0.40 ($p<0.01$) for the correlation between CRP levels at the start of the study and CRP levels two months later [Kendalls' tau]. In 92% of the patients, CRP levels were above 2 mg/l during at least one occasion, whereas in 68% of patients, CRP levels were above 10 mg/l during at least one measurement. In 96% of the patients with CRP levels above 10 mg/l, significant clinical events and/or chronic co-morbidity was observed. In contrast, in the 14 patients with CRP levels varying between 2 and 10 mg/l during the three occasions, only in 1 patient (7.1%) a clinical event became apparent.

Conclusion
This clinical survey showed, next to a very high incidence of acute and chronic co-morbid events in dialysis patients, a large variation in CRP levels during a three months follow-up period. In nearly all patients, CRP levels above 10 mg/l were associated with intermittent clinical events or severe co-morbidity, which could easily be detected by physical examination and simple additional tests.

Introduction

In recent literature, a strong emphasis has been placed on elevated C-reactive protein (CRP) levels as an important risk factor for morbidity and mortality in HD patients[1,2]. Moreover, a relation has been observed between elevated CRP levels, atherosclerotic plaques and malnutrition (the MIA syndrome)[3]. Nevertheless, clinical impressions in our dialysis unit, we firstly noted a large variability in CRP-levels and furthermore had the feeling that changes in CRP levels were highly related to intermittent or chronic (co-)morbidity. In the literature, data on the relation between changes in CRP levels and intermittent morbidity are scarce[4], as are data on the variability of CRP levels per se in hemodialysis patients. Aim of the present study was to observe the variability of CRP levels and also to make an attempt to link elevated CRP levels to intermittent clinical events.

Methods

CRP levels (non-sensitive assay Syncron LX 20®, Beckham Coulter, CA; cut-off point 2 mg/l) were assessed during a monthly interval in all hemodialysis patients of our unit (n=60, mean age 68 ± 10 years, 28 males, 32 females) during a three months period. Moreover, all patients were seen weekly on clinical rounds during which special emphasis was placed on intermittent clinical events during the weekly period. If necessary, clinical rounds were followed by additional laboratory, bacteriological, and radiological examinations, based on the complaints of the patients. The correlation between the CRP levels at the three time periods was assessed by Kendall's tau.

Results

Median CRP-levels at month 0,1 and 2 were respectively 11 mg/l (range 2-394 mg/l), 9 mg/l (range 2-176 mg/l), and 9 mg/l (range 2-565 mg/l). The non-parametric correlation coefficient between logCRP levels at the start of the study and one month later was $r=0.55$ ($p<0.01$) and 0.40 ($p<0.01$) (figure 7.1). In 92% of the patients, CRP levels were above 2 mg/l during at least one occasion, whereas in 68% of patients, CRP levels were above 10 mg/l during at least one measurement.

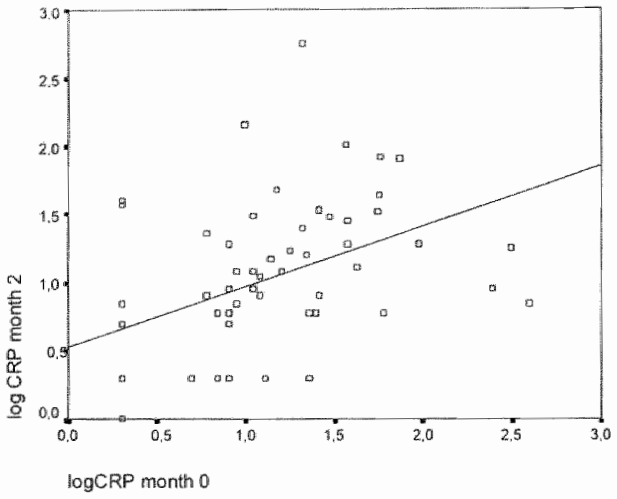

Figure 7.1 Relation between logCRP levels at the start of the study and two months later.

In 96% of the patients with CRP levels above 10 mg/l, significant clinical events and/or chronic co-morbidity was observed [acute or chronic inflammatory events (61%), malignancies (7.3%), recent surgery (4.9%), fractures (7.3%), symptomatic coronary artery disease (4.9%), active vasculitis (2.4%), gastrointestinal bleeding (2.4%)]. In contrast, in the 14 patients with CRP levels varying between 2 and 10 mg/l during the three occasions, only in 1 patient (7.1%) a clinical event became apparent.
Three patients died during the study period (5%) (1 myocardial infarction, 1 postoperative after aortic valve replacement and 1 of terminal multiple myeloma), whereas 11 patients (18.3%) were admitted to the hospital ward.

Discussion

The present data are in line with a recent observational study performed by the HEMO group, which showed a large variation in CRP levels in dialysis patients[4]. Nevertheless, in this study, variations in CRP levels were not related to clinical events.
A question which remains regards the cause of borderline elevated CRP levels. Although it cannot be proven from the present data, earlier studies showed that subclinical infections with e.g., chlamydia pneumoniae or helicobacter pylori were associated with mildly elevated CRP levels in dialysis patients[5,6].

Moreover, a recent study showed a relation between elevated CRP levels and silent ischemic heart disease in dialysis patients[7].

Concluding, this preliminary clinical survey showed, next to a very high incidence of acute and chronic co-morbid events in dialysis patients, a large variation in CRP levels during a three months follow-up period. In nearly all patients, CRP levels above 10 mg/l were associated with intermittent clinical events or severe co-morbidity, which could easily be detected by physical examination and simple additional tests. In patients with CRP levels between 2 and 10 mg/l, the association with clinical events was not clear. CRP levels above 10 mg/l in dialysis patients warrant a thorough clinical examination in dialysis patients.

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Chapter 8

Summary and conclusions

Summary and conclusions

Technical aspects of renal replacement therapies

The most commonly used renal replacement techniques are hemodialysis and peritoneal dialysis. During hemodialysis, blood is cleansed mainly by diffusion, due to concentration differences between the blood and a purified solution containing a balanced mix of electrolytes and buffer (dialysate), separated by a semipermeable artificial membrane. In this way, the blood is cleansed of smaller molecular weight uremic toxins, and electrolyte and acid-base abnormalities are corrected. During peritoneal dialysis, the main clearance principle is also diffusion, based on concentration differences between blood in the peritoneal capillaries and a purified solution installed in the peritoneal cavity, which are separated by the peritoneal membrane. Whereas hemodialysis is an intermittent therapy (usually performed 3 times weekly for 3-5 hr), peritoneal dialysis is a more or less continuous technique. However, the clearance per unit of time during peritoneal dialysis is far smaller compared to hemodialysis and therefore, weekly solute clearance does not exceed that of hemodialysis. During hemodialysis, blood flows at a rate of 250-400 ml/min through the extracorporeal circuit, consisting of blood lines and a artificial membrane. The latter consists of a multitude of semipermeable fibres, which separate the blood from the dialysate compartment, through which a purified solution is running at a rate of ±500 ml/min. Earlier, these fibres were predominantly made of cellulose, however due to the activation of the immune and clotting system (bio-incompatibility) with the use of unmodified cellulose membranes, the use of synthetic membranes has greatly increased.

Whereas diffusion is an efficient process for the removal of small molecular weight uremic toxins, the removal of toxins of a larger molecular weight is limited by the nature of the diffusive process itself, as the random movement on which diffusion is based, is slower for larger than for smaller molecules. Moreover, the most commonly used dialysers for hemodialysis have a relatively small pore size and thus transport of larger molecules is limited. Although, apart from the diffusive process, also a pressure gradient over the dialyser is generally needed to remove the excess fluid (ultrafiltration), this will not contribute to a significant larger molecular weight clearance due to the small volumes and low permeability of the dialyser. Thus, the removal of so-called middle molecular (500-5000 D) and especially large molecular weight >5000D) is limited during conventional hemodialysis treatments. This is important because the normal cut-off point for solute removal of the glomerulus is approximately 60kD. Thus, there remains a wide spectrum of uremic toxins

which is not removed by conventional hemodialysis (or peritoneal dialysis) and which are accumulated in dialysis patients. An important example of such a toxin is beta-2 microglobulin (ß2M), which plays an important role in the pathogenesis of dialysis related amyloidosis, characterized by cartilage destruction and carpal tunnel syndrome.
A mechanism to overcome this problem is by using convection (i.e., transport based on pressure differences) as the mechanism for solute clearance, which is also the way by which the normal kidney operates. Renal replacement modalities based on convection can be performed without (hemofiltration) or with concomitant diffusion (hemodiafiltration). During hemofiltration, plasma water is filtered through a highly permeable artificial membrane, and substituted by a sterile (substitution) solution with a composition approximating that of dialysate. The amount of blood which has to be filtered during hemofiltration to achieve an acceptable clearance is large. The use of prefabricated substitution solutions made hemofiltration a very expensive treatment also in view of the large and highly permeable membranes needed. Moreover, when infusing substitution fluid in the postdilution mode (i.e., after the blood has passed the dialyser), significant hemoconcentration occurs within the artificial membrane where the blood is filtered. In general, not more than 25-30% of blood flow can be filtered in post-dilution mode, which yields a filtration volume of ±100 ml/min, or ±24 litres per treatment. However, a filtration volume approximating the urea distribution volume (i.e., approximating total body water or 60% of the patients body weight) is needed to achieve an adequate small molecular clearance. All these factors led to an abandonment of hemofiltration as a routine therapy in the late eighties and early nineties. However, with the introduction of so-called on-line filtration techniques, by which the substitution fluid is produced from pretreated water and dialysate concentrates which are further cleansed by ultrafilters that reject and absorb endotoxin and bacterial fragments, it became possible to produce large amounts of substitution fluid in an economical feasible way[1]. However, the introduction of so-called on-line filtration therapies had to take some hurdles. As in the Netherlands, the substitution fluid is considered a pharmaceutical, it has to apply to the demands of the European Pharmacopoea[3]. Moreover, it should be kept in mind that with the dialysis modules suitable for on-line filtration techniques, up to 24 litres of substitution fluid per hour can be infused. With the availability of high volumes of substitution fluid, it became possible to perform hemofiltration in pre-dilution mode (i.e. the substitution fluid is infused before the artificial membrane). The dilution of the blood results in lesser hemoconcentration and thus to the possibility of higher filtration volumes. However, due to the dilution of the blood (mostly in a 1:1 ratio between blood and substitution fluid), twice the urea distribution volume, or (as a rule of thumb,) 1.1-1.2 times the body weight has to be filtered per treatment to

achieve adequate small molecular clearances. Due to the high permeability and large surface areas of the membranes used for pre-dilution on-line hemofiltration, this target is generally approximated, although, a slightly lower small molecular weight clearances are achieved during on-line hemofiltration compared with low-flux hemodialysis[2]. Hemofiltration can also be performed together with hemodialysis and is then called hemodiafiltration. Hemodiafiltration offers the best combination of smaller and larger uremic toxins, and the filtration volume becomes less of a problem as small molecular clearance is guaranteed by concomitant dialysis. In general, due to the additive effect of convection and the use of artificial membranes with large surface areas, also small molecular clearance is generally higher compared to conventional hemodialysis. However, in general, larger molecular clearance is less compared to (pre-dilution) hemofiltration[1]. This is due to the fact that hemodiafiltration is mostly performed in post-dilution mode, as pre-dilution infusion of substitution fluid leads to a fall in the concentration gradient needed for diffusion[1].

Higher larger molecular weight clearance can also be achieved with hemodialysis using highly permeable membranes (so-called high flux hemodialysis). However, larger molecular weight clearance is also hampered by the diffusive process per se (i.e., the larger the molecular weight, the slower the movement of the molecules). Although due to a pressure gradient within the dialyser, also a small amount of filtration (backfiltration) occurs in high-flux dialysers, larger molecular weight clearance will in general not approach the achievement of on-line hemo(dia)filtration and will in general not be higher than 25 ml/min[4].

Until now, the highest larger molecular weight clearances have been achieved with the use of so-called pre-dilution on-line hemofiltration.

In this thesis, hemofiltration was chosen as the therapy of choice to be compared with conventional hemodialysis, as in this way it was possible to study the effect of increasing larger molecular clearance with comparable small molecular clearance compared to low-flux hemodialysis. This could lead to a better understanding of the pathophysiologic importance of larger molecular weight uremic toxins.

Indeed, as will be discussed below, convective therapies have also been advocated for the prevention of intra-treatment and long term dialysis complications in patients on renal replacement therapy.

Short-term complications

The most cumbersome intra-dialytic complication is hypotension. It has been estimated to occur in 20% of treatments, although the incidence seems to have decreased during the last years due to improvements in dialysis technique.

The pathophysiology of dialysis-related hypotension is multifactorial. However, the three most important contributory factors are a decline in blood volume, an inadequate peripheral vascular resistance, and impaired cardiac function[5].
Various studies have reported a benefit of convective treatments on the prevention of intra-treatment hypotensive episodes[2,6]. For this phenomenon, different factors might be responsible.
It has been proposed that the decline in blood volume, which is the resultant of fluid removed during the dialysis treatment, and the refill from the interstitial tissues, is less pronounced during convective treatments compared to standard hemodialysis. This could be due to an enhanced Donnan effect due to increase coating of (negatively loaded) proteins to the artificial membrane, thus impairing sodium removal, which leads to an increase shift of fluid from the intra- to the extracellular spaces[7]. However, in contrast to studies by Locatelli et al.[8,9], who reported an increased Donnan effect during pre-dilution hemofiltration, we did not observe differences in sodium removal between hemodialysis and pre-dilution HF, as reported in chapter 3[9]. Differences between studies might be caused by the fact that in our approach higher filtration volumes (and thus higher infusion volumes of substitution fluid) are used in pre-dilution mode, leading to less pronounced Donnan effect. Moreover, as described in chapter 3, no differences in blood volume decline were observed, in agreement with earlier data from van Kuijk[10].
Another important contributory factor in the pathogenesis of intra-dialytic hypotension is an inadequate constriction of resistance and capacitance vessels during a decline in blood volume. An inadequate reduction of resistance vessels (arteries and arterioles) will directly lead to a reduction in blood pressure, being the product of cardiac output and systemic vascular resistance. An inadequate constriction of the capacitance vessels (veins and venules) will lead to less centralisation of unstressed blood volume from the peripheral tissues, resulting in a decline in central blood volume (blood located in the heart and great blood vessels), and thus in cardiac output[5]. Earlier studies showed a clear improvement in the vascular response during hemofiltration compared to hemodialysis[11]. It has been suggested that an increased removal of vasodilating substances, like calcitonine related gene peptide, might be responsible for this phenomenon[12]. However, direct proof for this hypothesis is still lacking. Studies from the different groups, including ours, have shown the overwhelming influence of thermal factors on the vascular response during renal replacement techniques. During hemodialysis with a (commonly used) dialysate temperature of 37°C to 37.5°C, core temperature increases[13]. During conventional hemo(dia)filtration, significant extracorporeal blood cooling occurred because the prefabricated substitution fluid is not infused at body temperature[14]. However, even with on-line hemofiltration, blood cooling is larger compared to hemodialysis, even when the substitution fluid

has the same temperature as dialysate. This was shown in an in-vitro study in chapter 2, in which extracorporeal energy transfer was compared between various modifications of on-line convective therapies and hemodialysis treatments at different dialysate temperatures. It was shown that extracorporeal energy transfer was comparable between hemodialysis treatment with a dialysate temperature of 37.5°C and pre-dilution hemofiltration with an infusate temperature of 36.5°C[15]. The additional blood cooling during hemofiltration is likely due to heat loss from the artificial membrane, as no dialysate is running during this technique, and the infusion line.

Although it would be expected that blood cooling is less pronounced during on-line hemodiafiltration because the artificial membrane is a very effective heat exchanger, extracorporeal blood temperature was still lower during on-line hemodiafiltration compared to hemodialysis, possibly due to heat loss from the substitution line[16]. Increased extracorporeal blood cooling has a pivotal effect on the vascular response during renal replacement therapies. If the increase in core temperature is blunted, cutaneous vasodilation in order to remove the excess heat becomes unnecessary and the vascular response during fluid removal becomes physiological[17,18]. Interestingly, extracorporeal blood cooling might have vasoactive effects beyond the simple cutaneous vascular response to thermal stimuli. Earlier studies during hemodialysis treatment showed that an increased production of the endogenous vasodilator nitric oxide might be involved in hemodialysis hypotension. However, the activity of the enzyme inducible nitric oxide synthase is temperature dependent, as has been shown in studies in patients with experimental heat stress. Thus, it was hypothesized that extracorporeal blood cooling during hemodialysis treatment might also influence the synthesis of nitric oxide during hemodialysis. Indeed, during hemodialysis with a dialysate temperature of 37.5°C, both core temperature and nitric oxide synthetic capacity increased, in contrast to hemodialysis with a dialysate temperature of 35.5°C, during which nitric oxide synthetic capacity remained unchanged[19], as described in chapter 4.

Thus, although there is no doubt that increased blood cooling may at least be partly responsible for the improved vascular response during hemofiltration, there is still discussion whether the additional removal of potential vasodilating substances in the mid molecular range during hemofiltration has an additional beneficial effect on the vascular response during hemofiltration. Therefore, in chapter 3, the vascular response was compared between hemodialysis treatment with dialysate temperatures of respectively 35.5°C and 36.5°C, and pre-dilution on-line hemofiltration with an infusate temperature of 36.5°C, using the saline dilution technique. No differences in the vascular response between hemodialysis and hemofiltration at 36.5°C was observed, whereas the decline in central blood volume was even slightly larger during HD at a temperature of 35.5°C[9].

Summarizing, the results of the present thesis suggest that convective treatments do not have major additional effects on the hemodynamic response during dialysis treatments which go beyond thermal factors. This is in line with earlier studies of van Kuijk, who observed no difference in forearm vascular response and venous tone between hemodialysis and hemofiltration when both treatment modalities were matched for thermal factors[10]. However, vascular response in the study of van Kuijk was assessed using strain-gauge plethysmography, which measured the vascular response at a regional level, whereas in the present study the systemic vascular response was assessed by the saline dilution technique.

Effect of convective therapies on long-term complications

Patients on dialysis treatment have a greatly enhanced risk on mortality compared to the general population. There does not appear to be a major difference in age-adjusted mortality between patients treated with hemodialysis and peritoneal dialysis[20]. The main cause of mortality in hemodialysis patients is cardiovascular disease. Hypertension and structural abnormalities of the cardiovascular system, such as left ventricular hypertrophy, increased arterial stiffness and atherosclerosis are very common in dialysis patients[21].

The cause of the increased burden of cardiovascular disease is determined by multiple factors, such as hypertension, anemia, inflammation and disturbances in the calcium-phosphate product. However, it has also been hypothesized that accumulation of uremic toxins, which are inadequately removed by low-flux hemodialysis, might play a role in this phenomenon.

In renal failure, levels of l-asymmetric dimethylarginine (ADMA) are increased. ADMA is an endogenous inhibitor of nitric oxide synthase and has been associated with left ventricular hypertrophy, hypertension and structural abnormalities of the large arteries in patients with end-stage renal disease[22,23]. Regarding its relatively low molecular weight of 202 D, ADMA cannot be called a "true" middle molecular weight uremic toxin. However, the removal of ADMA during conventional hemodialysis is limited, as will be discussed later.

Moreover, it has also been hypothesized that accumulation of advanced glycation end products in the cardiac and vascular wall might also play a role in the pathogenesis of structural cardiovascular abnormalities in dialysis patients[24].

Regarding the effects of convective treatments on long-term complications, earlier cross-sectional studies and observational studies showed promising results in term of (long-term) outcome and cardiovascular mortality[25,26]. It should be noted that earlier studies comparing hemodialysis were not matched for biocompatibility of the artificial membrane. Moreover, in general, some kind of contamination of the dialysate can not be excluded. With regard to

hemodialysis, an inverse relation between middle molecular clearance and mortality was observed[27]. Furthermore, longer hemodialysis (e.g., three times weekly 8 hours) or more frequent dialysis sessions (e.g., five or six times weekly), during which middle molecular clearance is improved, are associated with improved cardiovascular structure and blood pressure control[27,28]. On the other hand, the results of the randomised HEMO study, in which low-flux and high-flux membranes (with a larger permeability for larger molecules) did not show an effect of membrane permeability on total outcome, although the mortality from cardiac disease appeared to be slightly decreased[29,30]. However, the increased middle molecular clearance obtained during so-called high flux dialysis is far less than can be achieved using pre-dilution hemofiltration. Preliminary data showed a reduction in left ventricular hypertrophy during convective therapies[31].

In the randomised study reported in chapter 5, we did not observe an improvement in blood pressure control during pre-dilution on-line hemofiltration compared with low-flux hemodialysis. Data regarding the effect of convective techniques on blood pressure regulation are conflicting. Whereas several studies even observed an increase in inter-treatment blood pressure with the use of convective therapies[2,32], others found no change[34], in one small study an improvement in blood pressure control was observed during on-line hemodiafiltration in diabetic patients[33]. From the reports of the literature, it appears unlikely that the same improvement in hypertension control can be achieved as observed during more frequent or longer hemodialysis sessions. Other factors than increased middle or larger molecular clearance might be responsible for the blood pressure control during the latter treatment modalities, such as a reduction in fluctuations in fluid status and an improvement in sodium removal[35].

As also described in chapter 5, no effect of pre-dilution on-line hemofiltration on cardiovascular structure was observed. Beyond the relatively small patient numbers and short follow-up period, the reason for the absence of an effect might be due to various factors. Firstly, as shown in chapter 6, the presence of structural abnormalities might be partly related to the origin of renal disease[36]. Thus, patients in whom renal failure is caused by atherosclerosis and diabetes mellitus, are already at a higher risk for cardiovascular disease due to the generalized disease process[37]. Moreover, earlier studies showed a high prevalence of structural cardiovascular abnormalities already at the start of dialysis treatment or even earlier in the course of renal disease, suggesting that in many patients, cardiovascular damage already develops during the period of chronic renal disease[38,39]. The intermittent nature of the therapy, also in the case of highly efficient convective therapies, precludes a normalisation of fluid status and calcium-phosphate product, which are highly important factors in the

pathogenesis of cardiovascular abnormalities and even predict mortality in dialysis patients[40,41].

Another phenomenon which is linked to mortality in dialysis patients is malnutrition. A reduced appetite plays an important role in the pathogenesis of malnutrition in dialysis patients. Although the mechanism behind this phenomenon remains as yet unclear, it has been hypothesized, based on experimental data, that accumulation of uremic toxins in the middle molecular range might have an appetite suppressing effect[42]. The same has been hypothesized for the increased serum concentrations of leptin in dialysis patients[43]. Indeed, small but significant beneficial effects of pre-dilution on-line hemofiltration on nutritional state were observed in chapter 5. Changes in serum leptin levels however did not appear to be responsible for this phenomenon. In contrast, Wizemann did not find differences in nutritional state between patients randomised to on-line hemodiafiltration or high-flux hemodialysis, although in their study, nutritional state was not assessed in detail[34]. Also the use of high-flux versus low-flux hemodialysis did not result in significant improvements in nutritional parameters[44].
Malnutrition and cardiovascular disease are also strongly linked to inflammation in dialysis patients. The incidence of systemic inflammation is greatly enhanced in hemodialysis patients and also is a strong risk factor for mortality[45]. The reason for the persistent inflammatory state in uremic patients is multifactorial and might include factors such as the renal failure per se and the dialysis treatment[45,46]. Especially in earlier years, hemodialysis with contaminated dialysate and so-called bioincompatible membranes had a powerful stimulatin effect on the immune system[46]. It has been suggested that on-line hemodiafiltration might reduce inflammation, on the other hand there has been concern that the infusion of large amount of substitution fluid might actually trigger an inflammatory response[47-49]. However, no signs of monocyte activation were observed during treatment with on-line hemodiafiltration[49], whereas, as described in chapter 5, no difference in C-reactive protein levels were observed between patients treated with low-flux hemodialysis and on-line hemofiltration.
Thus, it appears that with the use of purified dialysis fluid and so-called biocompatible artificial membranes (which gives less rise to activation of the immune and clotting system) the effect of renal replacement therapy on the inflammatory state of dialysis patients has lost much of its importance. In chapter 7 it was shown that, in agreement with the data of van Tellingen et al., that levels of C-reactive protein as a marker of inflammation were mainly linked to co-morbid events[50,51]. The greatly enhanced incidence of infectious complications in dialysis patients is a major point of concern which might be due to the immune dysfunction in uremic patients. Regarding this aspect, it is of

interest that levels of complement factor D, which inhibits neutrophil degranulation but also the alternative complement route, were found to decrease during on-line hemo(dia)filtration treatment[32], (chapter 5).

Effect of on-line convective therapies on the uremic toxicity profile

As mentioned earlier, in general it can be stated that post-dilution on-line hemodiafiltration improves the clearance of both small and large molecular weight substances, the latter in general to a smaller degree compared to pre-dilution on-line hemofiltration[1]. Mechanisms to combine the best of both techniques, by combining pre- and post-dilution hemodiafiltration, but suffer from increased complexity[52]. An interesting novel approach is mid-dilution hemodiafiltration, by which the substitution fluid is infused in the middle of an artificial membrane using a two-cartridge design. Using this approach, the advantages of pre- and post-dilution hemodiafiltration are combined. However, published data with this approach are still lacking.

As mentioned previously, an important larger molecular weight toxin is β2M (12 kD). Data regarding the effect of on-line filtration techniques on β2M levels are still conflicting[53]. In general it can be stated that the decline in β2M levels is related to the amount of convective clearance[32,54]. As described in chapter 5, a decline of more than 50% in interdialytic β2M levels during pre-dilution on-line hemofiltration was observed compared to low flux dialysis. Also beneficial effects of on-line convective therapies on plasma levels of other larger molecular weight have been observed. As mentioned above, complement factor D (24 kD) decreased during pre-dilution on-line hemofiltration (chapter 5), but also during on-line hemodiafiltration[32]. Moreover, plasma levels of homocystein decreased, in agreement with a study on so-called superflux hemodialysis (hemodialysis with highly permeable membranes)[55]. Somewhat disappointing were the results on interdialytic levels of leptin, l-ADMA levels and advanced glycation end products related fluorescence, which did not change significantly during pre-dilution on-line hemofiltration. It should be noted however, that even an increase in clearance may not result in a reduction of pre- or interdialytic levels of uremic toxins, if the distribution volume is large. An important example is serum phosphate, which cannot be normalised by three times weekly therapies, regardless of the modality used[9,56].

Data on ADMA , which is actually a relatively small molecule, are in contrast with the data of Schroder, who observed a decline in predialytic levels using high flux hemodialysis[33]. Data on the clearance characteristics of ADMA are scarce. Its removal during low-flux hemodialysis is far less than expected regarding its molecular weight. It has been hypothesized that either protein binding or different interdialytic kinetics play a role in this phenomenon[23]. Data regarding the effect of on-line hemodiafiltration on ADMA levels are awaited

with interest. The effect of on-line convective therapies on AGEs is also conflicting. Lin et al., who observed a decline in total AGE levels in patients treated with hemodiafiltration compared to low-flux dialysis[57]. However, Gerdemann et al. observed lower levels of the AGE product carboxymethyllysine in HF treated patients only in comparison with HD patients treated with standard (i.e., contaminated) but not with ultrapure dialysate[58]. These authors suggested that the purity of the dialysate c.q. substitution fluid might play a role in the formation of AGEs. Moreover, differences in methodology to assess AGE products may contribute to discrepant results between different studies[59].

Future of convective therapies

In our view, it appears unlikely that convective therapies are a panacea for the prevention and treatment of complications in patients with end stage renal disease. Factors such as cardiovascular disease and malnutrition are influenced by multiple factors, and not all can be influenced by the treatment modality. Moreover, the intermittent nature of hemo(dia)filtration precludes the complete normalisation of fluid status, calcium-phosphate product and other uremia-related factors. In this aspect, it is of importance that the greatest improvements in cardiovascular status so far have been achieved with either longer and more frequent hemodialysis sessions, during which a near normalisation of fluid status and (in the case of daily nocturnal dialysis) calcium-phosphate product is possible[28]. However, more frequent and/or longer hemodialysis sessions are not yet generally available, mainly due to logistic and financial reasons.

Nevertheless, convective therapies are certainly more efficient in correcting the uremic milieu than standard hemodialysis and preliminary data have shown encouraging effects on cardiovascular and nutritional state and quality of life. An item which has received relatively little attention so far is the potential effect of convective therapies on the immune dysfunction of dialysis patients. On the other hand, the cost aspects of on-line convective therapies are not to be neglected. Although on-line filtration therapies are far cheaper compared to conventional hemo(dia)filtration with prefabricated substitution fluid, they still are more expensive compared to (low-flux) hemodialysis, due to the application of larger and more permeable artificial membranes, the need for more elaborate water purification techniques and the ultrafilters needed to reject endotoxins and bacteria, and the necessity of additional microbiological surveillance. Thus, larger randomised trials are needed to definitely establish the role of on-line hemo(dia)filtration techniques in the routine treatment of patients with end-stage renal disease.

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Samenvatting en conclusie

Samenvatting en conclusie

Technische aspecten van nierfunctie vervangende therapie.

Bij patiënten met ernstig nierfalen is behandeling met een zogenaamde nierfunctie vervangende therapie noodzakelijk. De meest toegepaste vormen van nierfunctie vervangende therapieën zijn hemodialyse en peritoneaal dialyse. Tijdens hemodialyse wordt het bloed van de patiënt gezuiverd, voornamelijk op basis van het principe van transport op basis van concentratieverschillen van afvalstoffen (diffusie). Als gevolg van een concentratieverschil tussen het bloed van de patiënt en het dialysaat, van elkaar gescheiden door een semi-permeabel (half doorlaatbaar) membraan, diffunderen afvalstoffen uit de patiënt. Op deze manier worden afvalstoffen, met name kleinere moleculen, verwijderd en worden elektrolyt en zuur-base afwijkingen gecorrigeerd. Tijdens peritoneaal dialyse is het voornaamste zuiveringsmechanisme eveneens diffusie, nu gebaseerd op de concentratie-verschillen tussen het bloed in de capillairen van het peritoneum en de spoelvloeistof in de peritoneaalruimte, gescheiden door het peritoneum. Hemodialyse is een intermitterende behandeling (meestal driemaal per week gedurende 3 tot 5 uur), dit in tegenstelling tot peritoneaal dialyse welke een meer continue techniek is. De klaring per tijdseenheid is bij peritoneaal dialyse veel minder dan bij hemodialyse, daardoor ontlopen hemodialyse en peritoneaal dialyse elkaar weinig in totale klaring per week.
Tijdens hemodialyse stroomt bloed met een snelheid van 250-400 ml/min door het extracorporale circuit, bestaande uit bloedlijnen en een kunstnier. De kunstnier bestaat uit een groot aantal semi-permeabele vezels, die het bloed van de patiënt scheiden van het dialysaat. Het dialysaat is een vloeistof bestaande uit water met daarin opgeloste bestanddelen (mineralen, glucose en bicarbonaat), dat met een snelheid van ±500 ml/min door de kunstnier wordt geleid. Vroeger werden de vezels van de kunstnier gemaakt van cellulose, echter als gevolg van de activatie van zowel immuun- als stollingssysteem hadden deze kunstnieren veel nadelige effecten. Deze, ook wel genoemd, bioincompatibele kunstnieren zijn nu voor een groot deel vervangen door meer biocompatibele kunstnieren vervaardigd van synthetische moleculen.
Diffusie is een efficiënte manier om moleculen met een laag moleculair gewicht te verwijderen, moleculen met een groter moleculair gewicht worden echter in mindere mate verwijderd. Deze verminderde klaring van moleculen met een groter moleculair gewicht is deels het gevolg van het proces van diffusie zelf. In de eerste plaats omdat de willekeurige bewegingen van grotere moleculen minder zijn dan die van de kleinere moleculen. Daarnaast zijn de poriën van de

membranen van de gebruikte kunstnieren te klein voor een goede doorlaatbaarheid van de grotere moleculen en beperken ze daardoor de klaring van deze moleculen.
Naast het verwijderen van afvalstoffen wordt er middels dialyse het overmaat aan vocht uit de patiënt verwijderd (ultrafiltratie), hetgeen wordt bereikt door het genereren van een drukgradiënt binnen de kunstnier. Ultrafiltratie draagt echter niet bij aan de klaring van de grotere moleculen gezien het geringe volume en opnieuw de beperking middels de grootte van de poriën in de membranen van de kunstnier. Er kan dus gesteld worden dat de verwijdering van moleculen met een groter moleculair gewicht, ook wel middelgrote moleculen (molecuul-grootte van 500-5000D) en grote moleculen (molecuulgrootte >5000D) genoemd, beperkt is bij conventionele dialyse gebruikmakend van diffusie. Dit is in tegenstelling tot de werking van de normale nier, waarbij moleculen tot een grootte van 60kD kunnen worden verwijderd. Bij de conventionele hemodialyse (en ook bij peritoneaal dialyse) wordt dus een grote hoeveelheid aan, mogelijk toxische, moleculen niet verwijderd en deze zullen zich ophopen bij dialysepatiënten. Een voorbeeld van een dergelijk groot molecuul is beta-2 microglobuline (ß2M), wat een belangrijke rol speelt bij het ontstaan van dialyse gerelateerde amyloidose, leidend tot botafwijkingen en carpaal tunnel syndroom.
Een methode om deze grotere, toxische, moleculen wel te kunnen verwijderen is het gebruik maken van convectief transport (gebaseerd op drukverschillen). Convectie is ook de wijze waarop de glomerulus van de nier (afval)stoffen filtreert en zodoende de zogenaamde voorurine maakt. Nierfunctie vervangende therapieën die gebruik maken van convectieklaring zijn hemofiltratie en hemodiafiltratie. Bij hemofiltratie is er alleen sprake van convectieve klaring, bij hemodiafiltratie is er een combinatie van diffusie en convectieve klaring. Tijdens hemofiltratie wordt plasmawater van de patiënt gefiltreerd door een hoog doorlaatbaar membraan. Dit verwijderde plasmawater moet dan wel worden vervangen door een substitutievloeistof. Deze substitutievloeistof is steriel en heeft een samenstelling ongeveer gelijk aan die van dialysaat. Om een acceptabele klaring te kunnen bereiken is de hoeveelheid plasmawater dat moet worden gefilterd erg groot, met een navenant benodigde hoeveelheid substitutievloeistof. Als men gebruik maakt van geprefabriceerde substitutievloeistoffen maakt dit hemofiltratie een erg kostbare behandeling, losstaande van de meerkosten van apparatuur en benodigde hoog doorlaatbare (en duurdere) kunstnieren. Daarnaast is er het technische probleem van hemoconcentratie (indikken van bloed). Indien substitutie vloeistof wordt toegediend na de kunstnier (post-dilutie), zal het bloed binnenin de kunstnier indikken als gevolg van het verwijderen van plasmawater. In de praktijk blijkt dat er niet meer dan 25-30% van de bloedstroom kan worden gefilterd zonder dat er stolling binnen de kunstnier zal

optreden. Dit betekent voor een standaardbehandeling een filtratie volume van ±100 ml/min, oftewel ±24 liter per behandeling. Echter voor een adequate klein moleculaire klaring is er een filtratievolume nodig van ongeveer het ureum distributievolume, oftewel het totale lichaamswater (60% van het lichaamsgewicht van de patiënt). Al deze bovengenoemde factoren hebben er uiteindelijk toe geleid dat de praktijk van hemofiltratie als behandeling bij chronische dialysepatiënten eind jaren tachtig begin jaren negentig werd verlaten. Door de introductie van de zogenaamde on-line filtratietechnieken kwamen hemofiltratie en hemodiafiltratie weer in beeld. On-line technieken houden in dat er substitutievloeistof word bereid uit voorbehandeld water en dialysaatconcentraat, middels extra filtratie met ultrafilters, welke endotoxinen en bacteriële resten uitfiltreren. Op deze manier is het mogelijk om grote hoeveelheden substitutievloeistof te bereiden op een economische haalbare manier[1]. De introductie van on-line filtratietechnieken had in eerste instantie nog wat problemen te overwinnen. Substitutievloeistof werd en wordt beschouwd als een farmaceutisch product, onder verantwoording van een apotheker en had dus te voldoen aan richtlijnen van de Europese Pharmacopoea[3]. Door de toepassing van strikte richtlijnen bij het de on-line filtratietechnieken werd het mogelijk om tot 24 liter substitutievloeistof per uur te infunderen in de patiënt. Door de beschikbaarheid van deze grote hoeveelheden substitutievloeistof werd het derhalve haalbaar om pre-dilutie hemofiltratie toe te passen, dat wil zeggen infusie van de substitutievloeistof voor de kunstnier. De daardoor veroorzaakte verdunning van het bloed van de patiënt, dus minder hemoconcentratie, maakte het mogelijk om grotere filtratie volumes toe te passen. Een nadeel echter is de verdunning van het bloed (meestal een 1 op 1 verhouding tussen bloed en substitutie vloeistof) en daardoor een inadequate klaring van kleinere moleculen. Om dit te voorkomen is het nodig om 1.1 tot 1.2 maal het lichaamsgewicht als substitutievloeistof toe te dienen. Als gevolg van de hoge permeabiliteit en grotere oppervlakten van de gebruikte kunstnieren zijn deze grotere hoeveelheden substitutievloeistof bij pre-dilutie hemofiltratie ook goed mogelijk, alhoewel in vergelijking met conventionele hemodialyse er soms een iets mindere klein moleculaire klaring wordt bereikt[2]. Er bestaat daarnaast de toepassing van on-line hemodiafiltratie. Hemodiafiltratie lijkt de beste combinatie te geven van klaring van kleinere en grotere moleculen, de nadelen van de hemodilutie bij pre-dilutie hemofiltratie wordt deels opgevangen door de klein moleculaire klaring van de hemodialyse. Bij hemodiafiltratie is als gevolg van het extra convectieve transport naast het gebruik van kunstnieren met grotere oppervlakten de klein moleculaire klaring meestal hoger dan bij conventionele hemodialyse. De groot moleculaire klaring tijdens hemodiafiltratie is echter wel kleiner dan bij pre-dilutie hemofiltratie. Deze verminderde groot moleculaire klaring is het gevolg van het feit dat er bij hemodiafiltratie er meestal gebruik wordt gemaakt van post-dilutie substitutie

van vloeistof. Als men gebruik zou maken van pre-dilutie is er sprake van een verminderde concentratiegradiënt en zodoende een beperking van diffusie[1].
In dit proefschrift is er voor gekozen om pre-dilutie hemofiltratie als methode te vergelijken met conventionele, low-flux, hemodialyse. Op deze manier is het mogelijk om de effecten van toegenomen groot moleculaire klaring te vergelijken bij ongeveer gelijkwaardige klein moleculaire klaring. Mogelijk dat dit kan leiden tot een beter begrip van de importantie van de grotere moleculen bij dialysepatiënten aangaande morbiditeit en mortaliteit. In eerdere studies met convectieve therapieën, met name hemodiafiltratie, is er al nadruk gelegd op de mogelijke preventie van nadelige korte- en lange termijn effecten tijdens deze behandelingen.

Effecten van convectieve therapieën op korte termijn complicaties

De meest voorkomende intra-dialytische complicatie is hypotensie. Uit eerder onderzoek is gebleken dat dit voorkomt in ongeveer 20% van de behandelingen, alhoewel het voorkomen de laatste jaren lijkt af te nemen als gevolg van verbeteringen in de dialysetechnieken. De etiologie van dialyse gerelateerde hypotensie is multifactorieel (door meerdere factoren) bepaald. De drie belangrijkste factoren lijken te zijn: de afname in bloedvolume tijdens de dialyse, een inadequate vasculaire weerstand en een afgenomen cardiale functie [5]. Diverse studies met convectieve therapieën lieten een positief effect zien op intra-dialytische hypotensie [2,6]. Voor dit fenomeen zijn diverse factoren mogelijk verklarend. Er is de veronderstelling dat de afname in bloedvolume, hetgeen de balans is tussen de vochtverwijdering (ultrafiltratie) tijdens de dialyse enerzijds en de daaropvolgende vochttoename vanuit het interstitieel weefsel anderzijds verminderd is tijdens convectieve therapieën. Dit effect kan het gevolg zijn van een verminderd natriumtransport door toegenomen coating van negatief geladen eiwitten aan het membraan van de kunstnier (Donnan effect). Dit toegenomen Donnan-effect tijdens hemofiltratie (veroorzaakt door de hogere druk in de kunstnier) kan de natriumverwijdering tijdens hemofiltratie doen afnemen, wat weer leidt tot een toegenomen shift van vloeistof van de intra- naar de extracellulaire ruimtes[7]. Echter in tegenstelling tot andere studies[8,9] vonden wij in de studie aangaande de natriumverwijdering tijdens pre-dilutie hemofiltratie en standaard hemodialyse geen verschil, als beschreven in hoofdstuk 3[9]. De verschillen tussen de diverse studies kunnen mogelijk worden verklaard door het feit dat in onze studie hogere filtratievolumes en dus meer substitutie vloeistof is gebruikt, hetgeen kan leiden tot een minder uitgesproken Donnan-effect. Tevens werd er tijdens onze studie geen verschil gevonden in bloedvolume afname, conform eerdere data uit ons centrum[10].

Een andere belangrijke factor in de pathogenese van intradialytische hypotensie is een inadequate samentrekking van weerstands en capaciteitsvaten tijdens een daling in bloedvolume. Deze inadequate reactie van de weerstandsvaten (arteriën en venen) kan leiden tot een daling in de bloeddruk, welke het product is van de cardiac output en de systemische weerstand. De inadequate reactie van de capaciteitsvaten (venen en venulen) leidt tot een verminderde centralisatie van het bloedvolume vanuit de perifere weefsels, waardoor het centrale bloedvolume afneemt (het bloed gelokaliseerd in het hart en de grote vaten) en een daling zal optreden in de cardiac output[5]. In vroegere studies werd er een duidelijke verbetering gevonden in de vasculaire respons gedurende hemofiltratie in vergelijking tot standaard dialyse[11]. Diverse veronderstellingen zijn geponeerd om dit te kunnen verklaren, gedacht werd dat substanties met vaatverwijdende capaciteiten werden verwijderd middels hemofiltratie[12]. Echter een direct bewijs voor deze veronderstellingen zijn tot dusver niet geleverd. Studies van diverse groepen, waaronder de onze, geven een overtuigend bewijs voor thermale effecten op de vasculaire respons tijdens dialyse. Tijdens dialyse, met een standaard temperatuur van het dialysaat van 37°C tot 37.5°C, is er een stijging van de centrale temperatuur van de patiënt[13]. Tijdens conventionele hemo(dia)filtratie treedt er extracorporele (buiten het lichaam) bloedafkoeling op als gevolg van de infusie van geprefabriceerde substitutievloeistoffen[14]. Ook als de temperatuur van de substitutievloeistof gelijk is aan de temperatuur van het dialysaat is er nog steeds sprake van afkoeling van het bloed tijdens hemofiltratie. Dit werd aangetoond in een in-vitro studie, beschreven in hoofdstuk 2, waarbij extracorporele bloedafkoeling werd vergeleken tussen diverse dialyse-modaliteiten; conventionele zowel als on-line convectieve methoden op verschillende dialysaat- en substitutievloeistoftemperaturen. Het bleek dat de extracorporele thermale balans tijdens hemodialyse met een dialysaat-temperatuur van 37.5°C gelijk was aan de afkoeling tijdens pre-dilutie hemofiltratie met een infusietemperatuur van 36.5°C[15]. Het extra effect op de bloed afkoeling tijdens pre-dilutie hemofiltratie is waarschijnlijk het gevolg van energieverlies via de kunstnier, aangezien hier geen dialysaat doorloopt, en van energieverlies via de infusielijnen.

Toegenomen extracorporele bloedafkoeling heeft een zeer belangrijke invloed op de vasculaire respons tijdens dialysebehandelingen. Als de toename in lichaamstemperatuur wordt beperkt, zal minder cutane vasodilatie (vaatwijding in de huid) optreden om de overmaat aan warmte te kunnen verwijderen en zal er een fysiologische respons op volumeverlies optreden[17,18]. Naast de cutane vasculaire respons op warmte stimuli kan de thermale balans tijdens dialyse ook andere effecten hebben welke de hemodynamische respons tijdens dialyse kunnen beïnvloeden. In eerdere studies werd aangetoond dat tijdens hemodialyse behandelingen er een toegenomen productie was van nitric oxide,

een endogene vasodilatator, als factor bij hypotensie tijdens dialyse. De activiteit van het enzym nitric oxide synthase is temperatuurafhankelijk, zoals aangetoond in experimenten met warmtestress. De hypothese dat de extracorporele bloedafkoeling tijdens hemodialyse de synthese van nitric oxide kan beïnvloeden werd getest in hoofdstuk 4. Het bleek dat tijdens hemodialyse met een dialysaattemperatuur van 37.5°C, zowel de lichaamstemperatuur als de synthese van nitric oxide toenam, in tegenstelling tot hemodialyse met een temperatuur van 35.5°C. Bij de laatste behandeling bleef de nitric oxide synthese onveranderd[19].

Alhoewel er dus weinig twijfel is over de effecten van toegenomen bloedafkoeling tijdens hemofiltratie als verklaring voor de betere vasculaire respons, blijft de vraag of er een extra hemodynamisch effect is als gevolg van de klaring van potentieel vasodilaterende stoffen van midden moleculaire grootte. In hoofdstuk 3 werd de vasculaire respons vergeleken tussen hemodialyse met respectievelijk een dialysaattemperatuur van 35.5°C en 36.5°C en pre-dilutie hemofiltratie met een temperatuur van 36.5°C van de geïnfundeerde substitutie-vloeistof. Middels natriumdilutie (verdunnings) technieken werd er geen verschil in vasculaire respons gevonden tussen hemodialyse met een dialysaat-temperatuur van 35.5°C of 36.5°C en hemofiltratie met een substitutie-temperatuur van 36.5°C, wel was de afname in centraal bloedvolume iets minder tijdens hemodialyse met een dialysaattemperatuur van 35.5°C[9].

Samenvattend, de resultaten van de studies in dit proefschrift beschreven, tonen aan dat convectieve behandelingen geen belangrijk additioneel effect lijken te hebben op de hemodynamiek tijdens dialysebehandelingen, anders dan de genoemde temperatuurseffecten. Dit is geheel in overeenkomst met eerdere studies van ons centrum, waarbij er geen verschil werd gevonden in vasculaire reactiviteit en veneuze tonus van de onderarm, tussen hemodialyse en hemofiltratie als beide behandelingen werden gecorrigeerd voor temperatuurseffecten[10]. Wel moet daarbij de kanttekening worden gemaakt dat in deze eerdere studie gebruik werd gemaakt van plethysmografie en in de huidige studie van de natriumdilutie methode.

Effecten van convectieve therapieën op langere termijn complicaties

Patiënten met nierfalen en zeker zij die nierfunctievervangende therapie moeten ondergaan hebben een sterk verhoogde kans op overlijden vergeleken met de normale populatie. Er blijkt geen duidelijke verschil te zijn tussen patiënten die worden behandeld met hemodialyse of peritoneaal dialyse[20] . De belangrijkste oorzaak voor overlijden van hemodialyse patiënten zijn hart- en vaatziekten. Hypertensie (hoge bloeddruk) en structurele afwijkingen aan het

hartvaatstelsel, zoals linkerventrikel hypertrofie (verdikking van de linker hartkamer), toegenomen vaatstijfheid en atherosclerose komen frequent voor bij dialysepatiënten[21]. De oorzaak voor het toegenomen voorkomen van hart- en vaatziekten in deze populatie is door meerdere factoren bepaald. Hypertensie, anemie, infectie en stoornissen in de calciumfosfaatbalans zijn een aantal van de (bekende) factoren. Een hypothese is dat de ophoping van toxinen (grotere moleculen), die niet adequaat worden geklaard door de standaard low-flux dialyse, een rol spelen bij dit verschijnsel. Bij patiënten met nierfalen zijn verhoogde waarden gemeten van l-asymmetric dimethylarginine (ADMA), een endogene remmer van nitric oxide synthase. ADMA is geassocieerd met hypertensie, linkerventrikel hypertrofie en structurele vaatveranderingen bij patiënten met nierfalen, de verwijdering van ADMA tijdens standaard hemodialyse is beperkt[22,23]. Veel aandacht is er voor de rol van "advanced glycation end products" (AGE's) in de etiologie van hart- en vaatafwijkingen bij dialysepatiënten[24].

De effecten van convectieve dialysebehandelingen op langere termijn complicaties, als cardiovasculaire morbiditeit en mortaliteit bij dialyse lieten veelbelovende resultaten zien in eerdere cross-over en observationele studies[25,26]. Echter daarbij moet wel worden vermeld dat de vroegere studies gedaan zijn met minder biocompatibele kunstnieren, conform de standaard van die periode. Verder waren de kwaliteitseisen aangaande het dialysaat, qua contaminatie en samenstelling, anders dan de huidige strengere eisen. Een inverse relatie was aangetoond tussen de middel moleculaire klaring tijdens hemodialyse en mortaliteit[27]. Langere dialysesessies (driemaal per week gedurende 8 uur) waarbij de middel moleculaire klaring is toegenomen zijn geassocieerd met betere cardiovasculaire structuur en betere bloeddruk-controle[27,28]. Echter de resultaten van de HEMO studie, waarbij gerandomiseerd werd tussen low-flux en high-flux membramen (de laatste met een grotere permeabiliteit voor grotere moleculen en dus betere klaring van grotere moleculen) lieten geen effect zien op de totale uitkomst, alhoewel de mortaliteit als gevolg van hartziekten iets afnam in de high-flux groep[29,30]. De toegenomen middel moleculaire klaring tijdens high-flux hemodialyse is echter veel minder dan bij pre-dilutie hemofiltratie of andere convectieve behandelingen. Eerste uitkomsten bij convectieve behandelingen lieten bijvoorbeeld een afname zien in linkerventrikel hypertrofie, als uiting van hart- en vaatziekte[31].

In de gerandomiseerde studie, beschreven in **hoofdstuk 5**, werd echter geen verschil gevonden in bloeddrukcontrole tussen pre-dilutie hemofiltratie en low-flux hemodialyse. Overige resultaten uit studies met convectieve behandelingen blijken ook tegenstrijdig te zijn. Diverse studies lieten een toename zien in bloeddruk tijdens de periode tussen de dialyse sessies bij convectieve therapieën[2,32], anderen vonden geen verschil[34], terwijl in een

kleinere studie een verbetering in bloeddrukregulatie werd aangetoond bij dialyse patiënten met diabetes tijdens hemodiafiltratie[33]. Alle resultaten in aanmerking nemende lijkt het dat tijdens hemofiltratie dezelfde verbetering in bloeddrukregulatie kan worden bereikt als tijdens frequentere of langere dialysesessies. Andere factoren dan de toegenomen midden en grotere molecuulklaring lijken verantwoordelijk voor de bloeddrukcontrole tijdens deze langere en/of frequentere behandelingen, zoals verminderde fluctuaties in volumestatus en een verbeterde natrium verwijdering[35].
In de studie, beschreven in hoofdstuk 5, werd tevens geen effect van pre-dilutie hemofiltratie gevonden op andere cardiovasculaire parameters. Het intermitterende karakter van de dialyse behandeling, zowel standaard als de convectieve therapieën, houden in dat er geen normalisatie kan optreden van volume status en calciumfosfaatbalans, beiden belangrijke factoren in de etiologie van hart- en vaatziekten en zelfs direct gerelateerd aan mortaliteit bij dialyse patiënten[36,37].
Een deel van de structurele cardiovasculaire afwijkingen bij dialysepatiënten is echter mogelijk niet te beïnvloeden door de dialysetechniek. Er is reeds een hoge prevalentie van hart- en vaatziekten voor de start van de dialysebehandeling, suggererend dat structurele cardiovasculaire afwijkingen zich ontwikkelen tijdens de gehele periode van chronisch nierfalen[38,39]. In hoofdstuk 6 werd aangetoond dat, met name bij jongere patiënten, de etiologie van de nierziekte onafhankelijk is gerelateerd aan de arteriële vaatwandstijfheid. Deze was significant hoger bij patiënten met diabetes of gegeneraliseerde vaatziekte als oorzaak van de nierinsufficiëntie vergeleken met patiënten met een "primaire" nierziekte [40,41].
Ondervoeding, dan wel malnutritie, is een andere factor gerelateerd aan de mortaliteit bij dialysepatiënten, een afgenomen eetlust lijkt hierin een belangrijke rol te spelen. Ook hiervoor zijn nog geen sluitende verklaringen gevonden, uit experimentele data kwam wel naar voren dat de ophoping van uraemische toxinen, met name die van middel moleculaire grootte, een remming gaven van de eetlust[42]. Hetzelfde eetlust remmende effect is toegeschreven aan toegenomen serumconcentraties van leptine bij dialysepatiënten[43]. Kleine, doch significante, gunstige effecten op de voedingstoestand werden gevonden in onze studie. Veranderingen in leptine waardes bleken hier echter niet voor verantwoordelijk. In een andere studie werd echter geen verschil gevonden tussen patiënten gerandomiseerd voor hemodiafiltratie of high-flux hemodialyse aangaande voedingstoestand[34]. Ook bij een vergelijkende studie tussen high-flux en low-flux dialyse werd er geen significante verbetering gevonden in voedingsparameters[44]. Aangemerkt moet echter wel worden dat in de laatste studies de lichaamssamenstelling niet in detail werd gemeten.

Ondervoeding en het voorkomen van hart- en vaatziekten is duidelijk gerelateerd aan chronische ontsteking bij dialysepatiënten. De incidentie van systemische inflammatie is toegenomen bij dialysepatiënten en een belangrijke factor voor mortaliteit[45]. De oorzaak voor deze chronische inflammatoire toestand is eveneens multifactorieel, waaronder het nierfalen zelf en de dialyse behandeling[45,46]. Vooral in de beginjaren van de dialysebehandelingen waren gecontamineerd (verontreinigd) dialysaat en bioincompatibele membramen een belangrijke stimulans voor het immuunsysteem[46]. Er is verondersteld dat on-line hemofiltratie een daling zou kunnen geven van de inflammatie, echter de infusie van grote hoeveelheden substitutievloeistof zou eveneens kunnen bijdragen aan een stimulatie van de inflammatoire respons[47-49]. Tijdens on-line hemodiafiltratie werd er echter geen tekenen van monocytenactivatie aangetoond[49], als marker van inflammatie. In onze studie werd geen verschil gevonden in CRP waarden tussen on-line hemofiltratie en low-flux dialyse, suggererend dat er geen verschil is qua inflammatie tussen beide groepen.
Het lijkt erop dat door de introductie van betere zuivering van het dialysaat, leidend tot zogenaamd ultrapuur dialysaat en het gebruik van biocompatibele kunstnieren het effect van de dialysebehandeling op inflammatie heeft doen afnemen. In hoofdstuk 7 zien we dan ook, in overeenstemming met andere studies, dat de waarde van het C-reactive proteine (CRP), als ontstekings-parameter, met name gerelateerd is aan optredende co-morbiditeit[50,51]. Co-morbiditeit lijkt de belangrijkste oorzaak voor stijgen van de CRP, bij vrijwel alle patiënten was een CRP waarde boven de 10mg/l geassocieerd met een infectieuze dan wel andere oorzaak (bv operaties).
Hierbij moet worden aangetekend dat de toegenomen incidentie van infectieuze problematiek bij dialysepatiënten een groot probleem is. Dit zou kunnen worden veroorzaakt door storing in het immuunsysteem bij uraemie. In dit aspect is het ook van belang dat de concentratie complement factor D, remmer van neutrofielen degranulatie maar ook van de alternatieve complementroute, afnam tijdens on-line hemo(dia) filtratie en bij onze studie[32]. De effecten van hemofiltratie op de immunologische reacties moeten echter nog worden onderzocht.

Effecten van convectieve therapieën op het uraemisch toxisch profiel

Bij standaard dialyse worden met name de kleinere moleculen verwijderd en in veel mindere mate de middelgrote en in het geheel niet de grotere moleculen. Tijdens hemofiltratie worden de middel grote en grotere moleculen beter verwijderd dan bij standaard dialyse, echter de kleinere moleculen worden bij hemofiltratie minder goed verwijderd in vergelijking met standaard dialyse. Tijdens hemodiafiltratie, de combinatie van hemofiltratie en standaard dialyse,

worden zowel kleine, middelgrote en grotere moleculen verwijderd, wel worden de grotere moleculen in mindere mate verwijderd dan in vergelijking met hemofiltratie[1].
Een belangrijk midden molecuul is beta-2-microglobuline (β2M) met een grootte van 12 kD. Effecten op de β2M-klaring van de on-line convectieve dialyse technieken zijn tegenstrijdig[52]. Gesteld kan worden dat de afname in β2M waarden is gerelateerd aan de mate van convectieve klaring[32,53]. Zoals beschreven in hoofdstuk 5, werd er een daling van meer dan 50% van de β2M serumwaarden aangetoond gedurende pre-dilutie hemofiltratie in vergelijk met standaard low-flux dialyse. Ook daling van andere groot moleculaire moleculen werd aangetoond tijdens convectieve behandelingen. Complement factor D (24 kD) nam af tijdens pre-dilutie hemofiltratie, maar ook bij on-line hemodiafiltratie[32]. Plasma waarden van homocysteine daalden tijdens convectieve behandeling, conform de observaties in studies met super-flux (gebruik makende van hoog permeabele membramen) kunstnieren[54]. Andere markers als leptine, ADMA en AGE's daalden niet tijdens de behandeling middels pre-dilutie hemofiltratie. Hierbij moet wel worden opgemerkt dat een toename in klaring niet perse hoeft te zijn gerelateerd aan een daling in serum waarden voor of na dialyse. Als het distributievolume groot is, zoals bij serum-fosfaat, is het vrijwel niet mogelijk om een normalisatie te bereiken bij driemaal per week dialyse, onafhankelijk van welke modaliteit er dan ook wordt gekozen[9,55].

De toekomst van convectieve therapieën

In deze studie werd bij behandeling met on-line hemofiltratie een toename van de kwaliteit van leven en een (lichte) verbetering van de voedingstoestand gevonden. Tevens werden er sterke verbeteringen in het uremisch toxinen profiel gevonden. Een significant effect op structurele cardiovasculaire afwijkingen werd echter niet gevonden. Complicaties als hart- en vaatziekten, ondervoeding worden beïnvloed door meerdere factoren, die niet kunnen worden opgelost door het alleen veranderen van de dialysemodaliteit. Daarnaast is hemo(dia)filtratie, net als standaard hemodialyse, een intermitterende therapie, wat als gevolg heeft dat er geen sprake kan zijn van een complete normalisatie van vochthuishouding, calciumfosfaatbalans en andere factoren van belang bij chronisch nierfalen. Het lijkt er op dat grotere verbeteringen op het gebied van hart- en vaatziekten kunnen worden bereikt door langere en frequentere hemodialyse, waarbij wel een bijna normalisatie van de vochthuishouding kan worden bereikt en in het geval van dagelijkse (nachtelijke) dialyse ook een normalisatie van de calciumfosfaatbalans[28]. Echtere deze langere en frequentere hemodialyse sessies zijn nog niet

algemeen haalbaar, voornamelijk als gevolg van logistieke en financiële redenen.
Convectieve technieken zijn wel meer efficiënt gebleken in het corrigeren van het uraemische milieu, door de klaring van moleculen met grotere moleculaire grootte, in vergelijking met de standaard hemodialyse.
Een ander aspect, wat weinig aandacht kreeg tot nu toe, is het mogelijke effect van convectieve therapieën op de immunologische disfunctie bij dialyse-patiënten.
Daarnaast moet het kosteneffect van convectieve therapieën niet worden onderschat.
Grotere, gerandomiseerde studies zijn nog steeds nodig om de plaats van convectieve therapieën als hemo(dia)filtratie bij de routine behandeling van patiënten met eindstadium nierfalen te bepalen.

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Dankwoord

Dankwoord

Zoals in de meeste proefschriften word vermeld, is het dankwoord een van de meest gelezen onderdelen en misschien daardoor ook het moeilijkste om te schrijven.
Toch waag ik hier een poging om een ieder te bedanken, die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift. Daarnaast wil ik alle personen bedanken die op welke wijze dan ook bijgedragen hebben aan mijn vorming en opleiding.

In de eerste plaats wil ik iedereen bedanken, die hieronder niet genoemd zullen worden. Sommigen omdat ik ze domweg in de loop van de tijd vergeten ben, waarvoor mijn welgemeende excuses. Anderen omdat dit dankwoord anders veel te omvangrijk wordt, allen bedankt.

Ik wil alle patiënten en hun families bedanken, die mee hebben willen werken aan de studies die in het kader van dit onderzoek zijn verricht. Het is zonder uw medewerking en toch ook opoffering niet mogelijk geweest om onderzoek op een dialyse afdeling te doen. Ik waardeer het ten zeerste dat u allen tijd heeft willen vrijmaken voor de diverse onderzoeken. Het is mijn hoop dat dit proefschrift, maar ook andere onderzoeken die nog lopen dan wel zullen worden verricht in de toekomst, er toe zullen bijdragen dat uw leven als dialysepatiënt zal verbeteren.

Ik wil mijn promotores, Prof. dr. K.M.L. Leunissen en Prof. dr. G. Remuzzi bedanken voor de mogelijkheid om dit onderzoek te volbrengen en hun bijdrage in de uiteindelijke totstandkoming ervan.

Mijn copromotor Dr. J.P. Kooman, beste Jeroen, zonder wiens hulp dit proefschrift nooit tot stand was gekomen. Dit proefschrift is zondermeer voor een deel jouw werk, naast de tijd en aandacht die je er aan hebt moeten en willen besteden, heb je het een en ander met me te verduren gehad. Ik hoop dat ik je in de toekomst hier en daar kan helpen en kan bijstaan als minimale tegenprestatie voor hetgeen je de afgelopen jaren voor mij hebt gedaan.

De leden van de beoordelingscommissie Prof. dr. R. Donckerwolcke, Prof. dr. M.P. van Dieijen-Visser, Prof. dr. C. Ronco, Dr. J.H.M. Tordoir en Prof. dr. R. Vanholder wil ik bedanken voor hun beoordeling van het manuscript. Ik beschouw het als een grote eer dat u daar de moeite voor hebt willen doen.

De dialyseafdeling van Vie Curi te Venlo, medisch geleid door Dr. A.J. Luik en verpleegkundig door Dhr. M. Gaasbeek. Best Ton en Max bedankt voor de mogelijkheid om op jullie afdeling het onderzoek te kunnen uitvoeren. Ik waardeer het enorm dat jullie mij alle faciliteiten gaven hiervoor, heb altijd met veel plezier in Venlo gewerkt. Enorm getroffen door jullie gastvrijheid en medewerking. Alle verpleegkundigen, secretaressen en technische medewerkers bedankt voor het feit dat jullie ten allen tijde mij behulpzaam waren.

De dialyseafdeling van het academisch ziekenhuis Maastricht, mijn thuisbasis al die jaren. Ik zou mensen tekort doen als ik hier maar slechts enkelen bij naam zou noemen. Weet dat ik jullie allen bedank voor jullie medewerking niet alleen tijdens het onderzoek, maar ook tijdens de opleiding en de eerste jaren nadien. Met veel plezier kijk ik terug op de ochtendkoffie met Marleen, Marjo, Piet en Paul.

Dank aan de afdeling klinische chemie van zowel Venlo als wel van Maastricht. Voor jullie bereidwilligheid om al die buisjes bloed telkens maar weer te verwerken en in de diepvries te stoppen.

De afdeling dietiek van beide ziekenhuizen, Linda Hover en Leny Klaassen dank voor jullie medewerking, vooral het bijhouden en uitwerken van de voedingsdagboeken was een hele opgave.

Verder denk ik terug aan ons carpoolgroepje, jaren hebben we met z'n allen de afstand tussen Eindhoven e.o. en Maastricht afgelegd. Frank van der Sande, Willy van Kuijk, Marten Nijziel en Christine Verhoeven, ik hoop dat jullie ook terug kunnen kijken op een goede tijd, wel was het erg vroeg opstaan. Verder wil ik Frank speciaal bedanken voor zijn aandeel in de uitvoering van het onderzoek en natuurlijk mijn opleiding tot nefroloog.

De overige leden van de vakgroep nefrologie wil ik bedanken: Prof. dr. J.P. van Hooff, Maarten Christiaans, Elly van Duynhoven, John Dackus, Monique Mullens, Eugenie van de Ham, Etienne Maas en Anke Hendriks. Bedankt voor de fijne samenwerking tijdens de Maastrichtse jaren. Daarbij wil ik ook zeker niet vergeten verpleegafdeling D5, al die jaren heb ik erg prettig met jullie mogen samenwerken.

Verder dank voor de steun aan al mijn medeassistenten nefrologie: Pieter van Paassen, Dirk Scheepers, Liesbeth Hendrickx, Tom Dejagere, Hans Boots, Marielle Krekels, Marc Hermans, Martine Verhoeven en Jos van de Kerkhof.

Speciale dank aan Stijn Konings, voor zijn hulp bij het voorbereiden en uitvoeren van de diverse onderzoeken.

Dank ook aan de sponsor van het onderzoek: Gambro AB Sweden en hun vertegenwoordiging in Nederland. Special thanks to ms. I. Ledebo, dear Ingrid thank you for your cooperation and your kind remarks during and after the study period.

Tiny Wouters wil ik bedanken voor haar hulp en steun bij de laatste, en inderdaad zwaarste loodjes bij het afronden van dit proefschrift.

Dank ook aan de leden van mijn huidige maatschap, voor het mij de mogelijkheid te bieden om het proefschrift af te kunnen ronden. Speciale dank aan Paul Gerlag en Warmold van den Wall Bake voor de samenwerking en het geven van een postacademische nefrologische opleiding. Verder dank voor de fijne samenwerking aan Ronald Erdtsieck, JoJan Keuning, Art Vreugdenhil, Marie-Louise Verhulst, Jan-Willem Straathof en Jan Salemans, vanzelfsprekend hoop ik dat we op deze weg voort kunnen gaan.

Mijn paranimfen wil ik bedanken voor hun hulp. Suzan Jeuken-Mertens, speciale dank voor het helpen opzetten van de studie en je begeleiding van mij in de wondere wereld van de on-line dialyse technieken. Johnny Cheng, beste swa, bedankt voor al je steun al sinds onze gemeenschappelijke start in 1985 in Groningen, ik heb veel bewondering voor je organisatorische talenten en je sportmanschap.

Als een na laatste dank aan mijn familie en vrienden voor al hun hulp en steun de laatste jaren.

En als allerlaatste en belangrijkste natuurlijk wil ik noemen mijn eigen gezin. Lieve Monique, Milou, Isabelle en Juliëtte bedankt voor jullie geduld en steun.

Ik heb gezegd, zoals dat dan zo mooi heet.

Curriculum vitae

Curriculum vitae

Charles Herbert Beerenhout werd geboren op 15 augustus 1966 in Nieuw-Schoonebeek (Drenthe).

Na het behalen van het VWO diploma in Emmen startte hij in 1985 met de studie Geneeskunde aan de Rijksuniversiteit te Groningen. Co-schappen werden doorlopen aan het Medisch Spectrum Twente te Enschede.
In mei 1992 behaalde hij zijn artsexamen.

Nadien werkte hij als arts-assistent (niet in opleiding) op de afdeling interne geneeskunde van het Academisch Ziekenhuis te Groningen en de afdeling interne geneeskunde van (destijds nog) het Diaconessenhuis te Eindhoven.

In september 1996 begon hij met de opleiding tot internist in het Catharina Ziekenhuis te Eindhoven. Opleider aanvankelijk Dr. W. Breed, nadien Dr.S.J. Hoorntje en als mede-opleider Dr. B. Bravenboer.
Eind 2000 werd de opleiding afgerond in het academisch ziekenhuis Maastricht, opleider Prof. dr. H.F.P. Hillen. Aldaar werd de vervolgopleiding gevolgd voor nefrologie, opleider: Prof. dr. J.P. van Hooff.
Vanaf 1 augustus 2002 is hij geregistreerd als internist en vanaf januari 2003 ook voor het aandachtsgebied nefrologie.

Sinds augustus 2003 is hij werkzaam in de maatschap interne geneeskunde/maag-darm en leverziekten van het Maxima Medisch Centrum locatie Veldhoven.

Hij is gehuwd met Monique Hew, samen hebben ze drie dochters: Milou, Isabelle en Juliëtte.

Table 5.2 Cardiovascular parameters

	Hemofiltration			Hemodialysis				
	0	6	12 months	0	6	12 months	time	mode
LVMi (g/m^2)	127 ± 33	141 ± 36	131 ± 24	135 ± 34	133 ± 33	138 ± 32	ns	ns
LVEDD (mm)	49.3 ± 4.6	50.2 ± 6.3	49.8 ± 4.0	50.4 ± 5.2	49.3 ± 6.6	52.1 ± 7.3	ns	ns
PW-EDWT (mm)	9.7 ± 1.7	10.4 ± 1.6	9.7 ± 1.1	10.3 ± 1.5	10.3 ± 1.4	9.9 ± 1.1	ns	ns
PWV (m/s)	12 ± 5	11 ± 3	13 ± 5	12 ± 3	13 ± 3	13 ± 5	ns	ns
48-hours systolic BP (mmHg)	135 ± 18	135 ± 14	132 ± 14	132 ± 25	129 ± 23	131 ± 18	ns	ns
48-hours diastolic BP (mmHg)	76 ± 14	75 ± 11	70 ± 13	74 ± 10	74 ± 10	74 ± 9	ns	ns
Antihypertensive agents (no)	1.3 ± 132	1.5 ± 1.1	1.6 ± 1.0	1.5 ± 1.2	1.9 ± 1.5	1.7 ± 1.4	ns	ns
Antihypertensive score	131 ± 125	150 ± 122	169 ± 114	147 ± 124	197 ± 148	167 ± 143	ns	ns
Noradrenalin (nmol/l)	5.3 ± 2.3	4.5 ± 2.7*	3.3 ± 1.5*	7.6 ± 3.1	3.4 ± 2.4*	2.6 ± 1.1*	<0.01	ns

LVMi = left ventricular mass index; LVEDD = left ventricular end-diastolic diameter; PW-EDWT = posterior wall end diastolic wall thickness; PWV = pulse wave velocity; BP = blood pressure; IVCD = inferior caval vein diameter. *p<0.05 compared to baseline

Table 5.3 Nutritional parameters

	Hemofiltration			Hemodialysis				
	0	6	12 months	0	6	12 months	time	Mode
Body weight (kg)	66.1 ± 11.3	68.3 ± 11.0	67.9 ± 10.5	73.1 ± 11.8	73.1 ± 10.4	74.2 ± 11.7	ns	ns
ECW (l)	17.9 ± 3.3	18.4 ± 3.8	18.5 ± 3.9	18.6 ± 4.0	19.1 ± 3.1	19.8 ± 4.1	ns	ns
LBM (kg)	44.8 ± 9.5	45.5 ± 9.6	46.2 ± 9.6*	49.4 ± 9.2	50.4 ± 8.3	50.6 ± 8.8	<0.05	ns
Fat mass (kg)	19.0 ± 4.9	19.3 ± 5.1	18.7 ± 5.9	18.9 ± 4.8	18.5 ± 4.6	8.4 ± 4.7	ns	ns
IGF-1 (ng/ml)	195 ± 100	156 ± 70*	196 ± 107**	220 ± 93	186 ± 90	166 ± 71*	0.06	<0.05
SGA	5.9 ± 0.8	0.2 ± 0.6	5.9 ± 0.6	6.1 ± 0.5	5.8 ± 0.6	5.8 ± 0.8	ns	ns
Serum albumin (g/l)	37.4 ± 3.5	35.8 ± 3.9	36.8 ± 3.9	36.7 ± 3.7	38.2 ± 4.6	6.3 ± 4.5	ns	ns
NPCR (g/kg body weight)	0.90 ± 0.2	0.85 ± 0.1	0.91 ± 0.1	0.89 ± 0.1	0.85 ± 0.12	0.89 ± 0.20	ns	ns

ECW = extracellular water; LBM = lean body mass; IGF-1 = Insulin-like growth factor 1; SGA = subjective global assessment; NPCR = normalised protein catabolic rate. *p<0.05 compared to baseline; **p<0.05 compared to t=6 months